LIPID COMPOSITION DURING OVARIAN MATURATION OF THE SHRIMPS, PENAEUS CHINENSIS AND METAPENAEUS ENSIS

by

CHUNG Chi Kong, Arthur

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The Chinese University of Hong Kong

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Thesis Committee:

Dr. K. H. Chu (Supervisor)

Dr. K. H. Yung (Supervisor)

Dr. V. E. C. Ooi (Internal Examiner)

Dr. N. Y. S. Woo (Internal Examiner)

Dr. J. D. Castell (External Examiner)

Abstract

Changes in total lipids, lipid classes and fatty acid composition in the ovary, hepatopancreas and muscle of the shrimps, Penaeus chinensis and Metapenaeus ensis during ovarian maturation were studied. Five maturation stages were identified, based on the colour and size of the ovary.

As the gonadosomatic index increased during ovarian maturation, lipids were accumulated in the ovaries of the shrimps. Total lipids in the ovaries of Penaeus chinensis and Metapenaeus ensis accounted for 13% and 22% of the tissue dry weight in stage I (immature ovary), increased to 39% and 43% in stage IV (mature ovary), and eventually decreased to 17% and 26% in stage V (spent ovary), respectively. In the hepatopancreas, total lipids exhibited a decrease during ovarian maturation. The concentration of hepatopancreatic lipids in Penaeus chinensis and Metapenaeus ensis decreased from 41% and 40% in stage I to 29% and 19% in stage IV, respectively. Total lipids constituted less than 5% of muscle dry weight and remained relatively constant during maturation in the shrimps.

Dominant components of ovarian and hepatopancreatic lipids were triglycerides and polar lipids. The two components often constituted more than 50% of total lipids. From stage I to IV, the concentration of triglycerides in the hepatopancreas of Metapenaeus ensis decreased from 186

mg/g to 50 mg/g tissue dry weight while concentrations of polar lipids also decreased. During the same period, polar lipids and triglycerides in the ovary increased from 57 to 204 mg/g and from 21 to 112 mg/g. The concentrations of neutral lipid classes in muscle remained roughly constant during maturation. Similar changes of lipid classes also occurred in Penaeus chinensis.

The ovary, hepatopancreas and muscle of both shrimps always contained high levels of palmitic (16:0), palmitoleic (16:1), oleic (18:1), and eicosapentaenoic (20:5ω3) acids, often comprising more than 60% of total fatty acids. During ovarian maturation, the concentrations of saturated and monounsaturated fatty acids increased in the ovary, but decreased in the hepatopancreas. On the other hand, the concentrations of polyunsaturated fatty acids rose in both the ovary and the hepatopancreas. Since the shrimps are incapable of de novo synthesis of polyunsaturated fatty acids, their increase would be attributed to the increase in the dietary intake of these acids. Thus, polyunsaturated fatty acids appeared to be necessary for ovarian maturation. The concentrations of all fatty acids in muscle remained roughly constant during ovarian maturation.

Accumulation of total lipids, lipid classes and fatty acids in the ovary during ovarian maturation are concurrent with the decrease in hepatopancreatic lipids in P. chinensis and M. ensis. This finding indicates that lipids

are mobilized from the hepatopancreas to the ovary for oocyte maturation. However, the decrease in absolute amount of hepatopancreatic lipids accounts for only about 20% of the increment in ovarian lipids, indicating that lipid requirement of the ovary depends not only on lipid storage in hepatopancreas, but also immediate intake of dietary lipids in ovarian maturation.

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TABLE OF CONTENTS

List of tables

Chapter 1 General Introduction

Decapod crustaceans are important species in fisheries and aquaculture. The world production of crustacean culture was 612,000 tonnes in 1989 (New, 1991). Though it only contributed to 4% of total aquaculture production, crustacean production has increased for more than 100% since 1984. Penaeid shrimps constituted nearly 88% of crustacean culture in the world in 1989. Most penaeid culture is practised in Asian countries.

Although shrimp culture is flourishing, there are still many impediments to its future development. One of them is that the supply of shrimp for broodstock still depends much on wild seed stock, such as postlarvae captured from the wild, or wild impregnated, gravid females. Owing to overfishing, wild stocks of shrimp are being depleted. The natural habitat of shrimp is being threatened by pollution. And the increasing energy costs are adversely affecting shrimp fishery. Thus, many efforts have been put on the complete culture of penaeid shrimp so that the shrimps obtained from hatcheries can grow and spawn, independent on the wild stocks. There have been many attempts to attain successful maturation in various penaeid species in captivity. The methods used include the manipulation of endocrine and environmental conditions, such as using eyestalk ablation (Makinouchi & Primavera,

1987), hormonal injection (Yano, 1985, 1987), and regulation of water temperature and photoperiod (Laubier-Bonichon, 1978; Brown et al., 1980; Crocos & Kerr, 1986).

In shrimp culture, eyestalk ablation and supply of natural food, such as bloodworm and mollusks are widely used for stimulating ovarian maturation of the shrimp. Middleditch et al. (1980a) suggested that some fatty acids in bloodworm might stimulate ovarian maturation of the shrimp, Penaeus setiferus. The shrimps also depend exclusively on exogenous source for cholesterol and polyunsaturated fatty acids because decapod crustaceans are incapable of de novo synthesis of these substances (Zandee, 1967; D'Abramo & Lovell, 1991). Therefore the quality and quantity of dietary lipid may be of great importance in ovarian maturation.

During ovarian maturation, accumulation of large amount of lipids in ovary have been reported in various shrimp species (Gehring, 1974; Teshima & Kanazawa, 1983; Galois, 1984; Jeckel et al., 1989; Mourente & Rodriguez, 1991). Vitellin, the main component of egg yolk, is a glyco-lipo-carotenoprotein which consists of about 40% of lipids (Meusy & Payen, 1988). Moreover, lipid globules are present in the oocytes during ovarian maturation. Thus lipids play an important role in ovarian maturation as sources of energy and as cell constituents in the processes of spawning, embryogenesis, hatching and early development of crustacean larvae (Holland, 1978; Chang & O'Connor,

1983). The present study aims at elucidating the variation in lipid contents of the ovary, hepatopancreas and muscle during ovarian maturation of the shrimps, P. chinensis and Metapenaeus ensis. The understanding of lipid profile during maturation may provide clues to formulate a maturation diet for shrimp broodstock from onset of maturation to spawning.

Penaeus chinensis and Metapenaeus ensis are two commercially important species in China and Southeast Asia. P. chinensis, previously known as P. orientalis, is distributed along the coast of China and of considerable commercial importance in the Yellow Sea, East China Sea and Korean Bight, where it is trawled (Holthuis, 1980). It is now the most important penaeid species cultured in China. The production of P. chinensis was also the highest in the world in 1989, constituting nearly 33% of the marine shrimp production (New, 1991). Metapenaeus ensis is widely distributed, from Japan in the east, to India in the west, and to northern Australia in the south (Holthuis, 1980). It is one of the most important species in the shrimp catches in Malaysia, Philippines, Indonesia and Singapore. Recently, M. ensis has been extensively cultured in Southeast Asia, including Taiwan, Indonesia, Philippines, Thailand, Malaysia and Hong Kong.

These two species are important fishery species in Hong Kong waters. In Hong Kong waters, P. chinensis has a short spawning season in the winter. It may only spawn

once a year. On the other hand, M. ensis has a long spawning season from March to October (Cheung, 1964). It probably spawns several times a year.

This thesis comprises five chapters. Chapter 1 is a general introduction to the study. Chapter 2 is a literature review. In the literature review, ovarian maturation of decapod crustaceans is described (Section 2.1), and the factors affecting ovarian maturation will then be explained (Section 2.2). The role of lipids during ovarian maturation of decapod crustaceans is also reviewed (Section 2.3). The variations of lipid composition during ovarian maturation in shrimps, P. chinensis and M. ensis will be presented in Chapter 3 and 4, respectively. Chapter 5 lists the general conclusions of the study.

Chapter 2 Literature Review

2.1 Ovarian maturation of decapod crustaceans

The process of ovarian maturation in crustaceans has been reviewed by Adiyodi (1985), Charniaux-Cotton & Payen (1988), and Meusy & Payen, (1988). Ovarian maturation of penaeid shrimp and lobsters are also described by Dall et al. (1991) and Aiken & Waddy (1980), respectively. This part of the literature review is based on these reviews and selected recent articles.

2.1.1 Female reproductive system of decapod crustaceans

The female reproduction system in decapod crustaceans consists of paired ovaries, oviducts, gonopores, seminal receptacles and the thelycum. The paired ovaries may be distinct or united by the ovarian bridge. A pair of oviducts leads from the ovaries to the gonopores. The sperm are transferred in spermatophores and usually received in the thelycum. Thelycum is lacking in anomurans, palinurans, and astacurans, but present in brachyurans and natantians (Dall et al., 1991).

In natantians, such as penaeid shrimp, the paired ovaries are bilaterally symmetrical (Dall et al., 1991). They extend from the oesophageal region to the sixth abdominal somite. The ovaries consist of two anterior

lobes, 6-8 short lobes and two long posterior lobes. The two oviducts lead from the sixth lateral lobes to the genital openings. The ovaries of palinuran, are in the form of paired cylindrical rods (Aiken & Waddy, 1980). They lie dorsal and lateral to the alimentary tract, and are united by a transverse bridge underneath the heart. The ovaries extend from the anterior end of the stomach at about the level of the eyes, to the insertion of the abdomen or beyond. A short oviduct connects the ovary with the genital aperture on the coxa of the third legs.

In brachyurans, the ovaries lie dorsal to the hepatopancreas (Johnson, 1980). They extend to either side along the anterior margin of the cephalothorax. The ovaries run in a posteromedial direction to the cardiac stomach, and just posterior to the stomach. They are joined medially by a commissure. The posterior lobes pass posterodorsally and laterally. These lobes are applied dorsally to the seminal receptacles, which are sandwiched on either side between the lateral wall of the body cavity and the lateral part of the pericardium. The oviducts pass ventrally from the seminal receptacles to open on the ventral surface of the sixth thoracic somite.

Generally, the ovary is difficult to be observed in immature animal. While the ovary becomes mature, the size of the ovary becomes larger and the colour will change from transparent or creamy white to green or red, depending on the species. Histologically, the ovary consists of an outer

thin epithelium, a fibrous layer of underlying connective tissue, and an inner layer of germinal epithelium (King, 1948; Johnson, 1980). It does not contain obvious muscle fibres and there are zones of ovarian proliferation throughout the ovary.

2.1.2. Ovarian maturation

Although ovarian maturation is a continuous process, it is often divided it into different stages because it is convenient for biologists to investigate the development at each stage.

The simplest method to determine the ovarian maturation is based on the observation of the colour and size of the ovary through the exoskeleton of the animal. The observation relies much on experience and is not suitable for decapods which have thick and dark exoskeleton, such as crabs and lobsters. This method is mainly applied to the shrimp. For example, in the shrimp, Metapenaeus ensis, five stages of ovarian maturation are distinguished as follows (Yano, 1985):

Stage I:

The ovary is transparent, with no distinguishable outline;

Stage II:

The ovary is visible as a thin opaque line along the dorsal central axis;

Stage III:

The ovary is visible as a thick opaque line;

Stage IV:

The ovary is turgid and opaque. The outline is distinct.

Stage V:

The ovary is turgid, broad and densely opaque. The outline is distinct. Spawning is imminent.

Ovarian maturation may also be divided into different stages, based on the diameter, shape and colour of oocytes. Kerr (1969) can distinguish eight stages of ovarian development in the blue crab, Callinectes sapidus as bellow:

Stage I:

Ovaries smooth, weighing less than 1 g. Oocyte diameter is 50-60 μm.

Stage II:

Ovaries exhibiting convolute exterior, weighing less than 5 g. Oocyte diameter ranges from 140 to 180 μm.

Stage III:

Ovaries in a further state of enlargement, weighing from 5 to 10 g. Oocyte diameter ranges from 200 to 250 μm.

Stage IV:

Large convoluted yellow ovaries, weighing between 10 to 15 g. Oocyte diameter ranges from 250 to 330 μm.

Stage V:

Ovary extremely large, weighing over 20 g.

Stage VI:

Ovaries stretched and empty; external sponge bright orange.

Stage VII:

Ovaries stretched and empty; external sponge dull orange to brown.

Stage VIII:

Ovaries stretched and empty; external sponge grey.

Determination of the gonadosomatic index (GSI) is also used to assess the maturity of decapods (GSI = weight of gonad x 100/ body weight). Teshima and Kanazawa (1983) used the GSI to classify five stages of ovarian maturation in the shrimp, Penaeus japonicus:

Stage I:	GSI < 2.9%
Stage II:	2.9% < GSI < 4.9%
Stage III:	5.0% < GSI < 8.0%
Stage IV:	GSI > 8.0%
Stage V:	Spent ovary

The histological method is the most accurate method to assess the degree of maturity in decapod crustaceans but it is laborious and time-consuming. Ovarian maturation has been described histologically in the shrimps, such as Parapenaeus longirostris (Tom et al., 1987), Penaeus japonicus (Yano, 1988) and P. monodon (King, 1948; Tan-Fermin and Pudadera, 1989), in the crayfish Cambarus sp., Orconectes sp. and Procambarus sp. (Beams & Kessel, 1963), and in the crab Callinectes sapidus (Johnson, 1980). Yano (1988) distinguishes 10 stages of ovarian development in Penaeus japonicus, but most are actually substages of five major stages, which correspond to those described by Tom *et al.* (1987) in Parapenaeus longirostris. Tan-Fermin and Pudadera (1989) divided ovarian maturation of Penaeus monodon into four stages: previtellogenesis, vitellogenesis, cortical rod and spent stages.

At the cellular level, ovarian maturation is often divided into two stages: previtellogenesis (primary vitellogenesis) and vitellogenesis (secondary vitellogenesis). Details of these two basic stages vary among the diverse groups of crustaceans. In general, previtellogenesis is the stage in which the yolk materials have not yet been deposited in oocytes. This stage is characterized by little growth in egg diameter, and development of cytological features which prepare the cell for protein synthesis. Vitellogenesis is the stage in which the yolk materials are manufactured and deposited in

the oocytes. This stage is characterized by a massive increase in oocyte size and weight, and the development of cortical crypts (granules) or a chorion. The morphological details of these two stages are described as follows.

2.1.2.1 Previtellogenic stage

In this stage, the ovary is dominated by oogonia and primary oocytes which result from division of oogonia in the germinative zone of the ovary (Meusy & Payen, 1988; Tan-Fermin & Pudadera, 1989). This zone is formed by the undifferentiated gonad of young female. The whole structure resembles a network in which each oogonium is completely surrounded by mesodermal cells. This structure persists in the whole life of the female. Oogonial mitosis takes place exclusively in the germinative zone. After the oogonia leaving the germinative zone, they become primary oocytes with condensed chromosomes which appear in synaptonemal complexes at the ultrastructural level. Unlike many vertebrates, the production of primary oocytes continues throughout adult life in crustaceans (Quackenbush, 1991).

A layer of follicle cells develops around each oocyte towards the end of this stage and pushes the oocyte towards the periphery of the ovarian tube in penaeid shrimp (Anderson et al., 1984; Dall et al., 1991). Follicle cells

at this stage are rectangular or cuboidal in shape in Penaeus monodon (Tan-Fermin & Pudadera, 1989).

During previtellogenesis, ribosomes develop and an extensive rough endoplasmic reticulum arises (Quackenbush, 1991). The glycoproteins are accumulated in the rough endoplasmic reticulum (Beams & Kessel, 1962; Charniaux-Cotton, 1985). This process is called endogenous vitellogenesis. This is confirmed by the appearance of immunologically distinct primary yolk, a glycoprotein, in the eggs of the amphipod, Orchestia gammarella, during previtellogenesis (Charniaux-Cotton & Payen, 1988).

In the penaeid, Sicyonia ingentis, the oocytes develop reticular elements during a cisternal phase of previtellogenesis (Duronsolet et al., 1975; Anderson et al., 1984). Then in the platelet stage which bridges the transition to vitellogenesis, the oocytes increase in size and yolk spheres appear in the cytoplasm for the first time. Micropinocytosis activity also increases during this transition (Anderson et al., 1984; Meusy & Payen, 1988). The previtellogenic stage stops when the oocyte reaches a diameter typical of the species.

2.1.2.2 Vitellogenic stage

Vitellogenesis is the phase of maturation where vitellin, the major constituent of yolk, accumulates in the oocytes. Vitellin is a lipo-glyco-carotenoprotein. It

contains between 28 and 35% of lipids and 4.8% of sugars (Meusy & Payen, 1988). Its molecular weight is about 340,000 to 500,000 (Fyffe & O'Connor, 1974; Junera et al., 1977, cited in Adiyodi, 1985; Lui & O'Connor, 1977). The precursor of vitellin is believed to be vitellogenin, which is abundant in the haemolymph during ovarian maturation. Vitellogenin is also a lipo-glyco-carotenoprotein. The molecular weight of vitellogenin does not differ much from that of vitellin (Junera et al., 1977, cited in Adiyodi, 1985). The molecular weight of vitellogenin is about 397,000 in the amphipod, Orchestia gammarella. No immunological difference between vitellogenin and vitellin has been found in any species (Meusy & Payen, 1988).

The source of egg yolk is a controversial issue in crustaceans and appears to vary among different species. Yolk may be exclusively produced in the ovary as in crayfish, or by both ovarian and extra-ovarian tissues as in crabs and lobsters, or exclusively by extra-ovarian tissues as in isopods and amphipods (Quackenbush, 1991). In insects, yolk is exclusively produced in the adipocytes of the fat body (Downer & Laufer, 1983). In decapod crustaceans, several sites for yolk protein production have been proposed. These sites include the haemocytes of the haemolymph, the hepatopancreas, the follicle cells of ovary and subepidermal adipose tissues (Adiyodi & Subramoniam, 1983; Kerr, 1969; Paulus & Laufer; 1987; Tom et al., 1987; Charniaux-Cotton & Payen, 1988). In the shrimp, Penaeus

japonicus, the ovary appears to be the sole source of egg yolk proteins (Yano and Chinzei, 1987). The follicle cells which surround the oocyte are suggested to be the site of egg yolk protein production. In Parapenaeus longirostris, egg yolk protein were found in the ovary as well as a subepidermal adipose tissue (Tom et al., 1987). The subepidermal adipose tissue was suggested to be a potential site for yolk protein synthesis. In Penaeus vannamei and Uca pigilator, egg yolk protein synthesis was demonstrated in both ovarian tissue and the hepatopancreas, suggesting that they are the sources of exogenous egg yolk proteins (Quackenbush & Keeley, 1987; Quackenbush, 1989).

During the vitellogenic stage, chromatin materials appear to be evenly distributed in the nucleoplasm of the vitellogenic oocytes. Oogonia are fewer in number compared to the previous stage, indicating that more oogonia have developed into oocytes. At the onset of vitellogensis, each oocyte is surrounded by a follicle envelope which comes from a permanent tissue, called the follicle tissue (Charniaux-Cotton & Payen, 1988). These secondary follicle cells which have been laid are utilized again for setting up the new follicles.

Among the oocytes of the amphipod, Orchestia gammarella, a distinct tubular network was observed in the follicle cells (Charniaux-Cotton, 1985). This network may be responsible for transport of cellular materials. At the beginning of vitellogenesis, the follicle cells develop a

network of circumvoluted and anastomosed tubules that communicate with the extracellular space. This structure extensively increases the permeability of the follicular epithelium. Thus, vitellogenin may be rapidly transferred from the haemolypmh to the oocyte surface through the follicular epithelium, extracellular spaces and through the tubular network. At the end of vitellogenesis, the tubular network disappears. This phenomenon has also been reported in decapods such as the shrimps, Sicyonia ingentis (Duronsolet et al., 1975) and Macrobrachium rosenbergii (Jugan, 1985, cited in Charniaux-Cotton & Payen, 1988), and the lobster Homarus americanus (Tablot, 1981a).

As the yolk spheres are incorporated into the oocytes, the size of oocytes increases remarkably (Quackenbush, 1991). In the homarid lobster, the primary oocytes grow from 100 μm to 1200 μm or larger during vitellogenesis. In penaeid shrimp, the mature oocytes of 250 μm has grown from the primary oocyte of 50-70 μm.

In the oocytes of penaeid shrimp, distinct cortical granule precursors begin to develop at the end of vitellogenesis. These cortical granules precursors will eventually form the characteristic penaeid cortical crypts (Clark et al., 1984; Tan-Fermin & Pudadera, 1989; Yano, 1988). The appearance of cortical crypts is an indicator of imminent spawning. The cortical granules eventually fuse with the egg oolemma, and contribute to an egg jelly which surrounds a fertilized egg (Anderson et al., 1984; Clark et

al., 1984). In the homarid lobsters, a chorion forms around the oocytes after vitellogenesis is completed. The chorion is derived from follicle cells and appears to perform the same function as the penaeid cortical granules (Talbot, 1981b).

At maturation, the nucleus moves to the cytoplasmic membrane and undergoes its first meiotic division. The follicle cells, when remain in the ovary, separate from the oocytes. The final cytological event in oocyte development is germinal vesicle breakdown (Anderson et al., 1984). In decapod crustaceans, the germinal vesicle break down is antecedent to ovulation. This event is considered to be the end of the vitellogenesis phase, and it usually occurs soon before ovulation (Anderson et al., 1984)

2.2 Factors affecting ovarian maturation in decapod crustaceans

2.2.1. Endocrine control

Endocrine control of ovarian maturation in decapod crustaceans has been reviewed by Adiyodi (1985), Charniaux-Cotton & Payen (1988), and Meusy & Payen (1988). Quackenbush (1986, 1991) and Aiken & Waddy (1980) have also reviewed the literature on endocrine control of ovarian maturation in penaeid shrimps and lobsters, respectively. This part of the literature review is based on these reviews and selected recent articles.

2.2.1.1. Inhibitory control

In the eyestalk of decapods, there is an important endocrine organ called the X-organ-sinus gland complex. The X-organ, containing neurosecretory cells, is situated in the medulla terminals of the optic lobes. The sinus gland stores and releases by exocytosis materials derived from the X-organ (Panouse, 1943, cited in Meusy & Payen, 1988).

In penaeid shrimp farms, unilateral eyestalk ablation is commonly used to induce maturation in female shrimp (Makinouchi & Primavera, 1987). This acceleration of ovarian maturation results from the removal of the X-organ-sinus gland complex (Panouse, 1943, cited in Meusy & Payen, 1988) which presumably secretes gonadal inhibitory hormone (GIH). In decapod crustaceans, GIH appears to be responsible for the genital rest by inhibiting vitellogenesis (Charniaux-Cotton & Payen, 1988). GIH is a peptide hormone, the chemical nature of which has not been completely characterized. Its molecular weight (MW) has been found to vary among species. The GIH isolated from Cancer magister is thermostable, and dialysable, with a MW of 2000 daltons (Bomirski et al., 1981). In other species, the MW is higher. The MW of GIH in shrimp, Penaeus vannamei, is 3.3 kD while the MW in the lobsters, Panulirus argus and H. americanus, ranges from 7 to 8 kD (Quackenbush & Herrnkind, 1981; Quackenbush & Keeley, 1986; Soyez et al., 1987).

By injecting tritiated leucine to female shrimps, Palaemon serratus, Meusy et al. (1983, cited in Meusy & Payen, 1988) showed that the ablation of eyestalks triggers vitellogenin synthesis. The correlation between the onset of vitellogenesis and the appearance of oocyte endocytosis in eyestalk-ablated animals (Shade & Shivers, 1980) was further investigated recently. An in vitro study using vitellin conjugated with colloidal gold, indicated that GIH probably regulates endocytosis by inhibiting vitellogenin binding to oocyte membranes (Jugan & Soyez, 1985, cited in Charniaux-Cotton & Payen, 1988). This inhibiting action can be explained at the molecular level either by a specific binding of the neurohormone to vitellogenin, preventing its binding to the membrane receptor, or by a direct binding of the hormone onto this receptor (or to a receptor in its vicinity), thus preventing the binding of vitellogenin.

2.2.1.2. Stimulatory Control

2.2.1.2.1 Gonad stimulating hormone (GSH)

Otsu (1963) gave the first indication of a stimulatory control of vitellogenesis by substances secreted by thoracic ganglia. He observed precocious development of the ovaries in the crab, Potamon dehaani, after implantation of thoracic ganglia. This result has been confirmed in other decapods. Boiled aqueous extracts of thoracic ganglia from the fiddler crab, Uca pugilator,

stimulated vitellogenesis in both intact and destalked animal (Eastman-Rek & Fingerman, 1984). Takayanagi et al. (1986) demonstrated in vivo and in vitro that aqueous extracts of not only thoracic ganglia but also the brain enhances vitellogenesis in oocytes of the shrimp Paratya compressa. Implantation of thoracic ganglion from the mature females into normal or destalked immature females of the crabs Paratelphusa hydrodromous and Libinia emarginata lead to a precocious yolk deposition (Hinsch & Bennett, 1979).

To conclude, the existence of a gonad stimulating hormone, secreted by nervous cells, seems to be established. However, the chemical nature of this hormone, possibly a peptide, its precise origin and the mechanism of its action remain to be investigated.

2.2.1.2.2 Vitellogenin stimulating Ovarian Hormone (VSOH)

From the work on the amphipod Orchestia, Junera et al. (1977, cited in Charniaux & Payen, 1988) concluded that the ovary produced a factor which stimulates synthesis of vitellogenin. This substance was named vitellogenin stimulating ovarian hormone (VSOH). Presumably, synthesis of VSOH is suppressed by GIH and promoted by some gonad simulating hormones. Up to now, there have only been a few studies on VSOH in crustaceans and none of them involves decapods.

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2.2.1.2.3 Ecdysteroids

The Y-organs control moulting by secreting α-ecdysone, which is hydroxylated to the active hormone, 20-hydroxy-ecdysone, also called ß-ecdysone or 20-ß-hydroxyecdysone (Chang et al., 1976). These ecdysteroids are collectively referred to as moulting hormones (MH).

Ecdysteroids have a role in the reproduction of insects and it has been speculated that they may also be critical to crustacean reproduction (Laufer & Landau, 1991). In amphipods and isopods, ecdysteroids appear to be necessary for vitellogenesis, thus having a reproductive role, in addition to moulting. In the amphipod, Orchestia gammarellus, destruction of the Y-organ at the beginning of the moult cycle results in no ovarian growth, and if the Y-organ is destroyed during ovarian growth the synthesis of vitellogenin stops (Meusy & Charniaux-Cotton, 1984). The Y-organ also seems to be required for oocyte growth in the isopod, Armadillium vuglare (Suzuki, 1986). In the crab, Callinectes sapidus, ovaries of mature females accumulated ecdysteroids during vitellogenesis (Soumoff & Skinner, 1983).

In contrast, ecdysteroids produces yolk droplet degeneration with a consequent decrease in the number of yolk droplets in the oocytes of barnacles (Fyhn et al., 1977). Likewise, injection of ecdysteroids into shrimp, Lysmata seticaudata, just after ecdysis inhibits

vitellogenesis (Touir & Charniaux-Cotton, 1974, cited in Meusy & Payen, 1988). The function of ecdysteroids on vitellogensis is still obscure.

2.2.1.2.4 Juvenoids

In insects, the juvenile hormone (JH) secreted by corpora allata regulates metamorphosis and gametogenesis. Thus, this juvenile hormone is suspected to play a similar role in reproduction of crustaceans (Meusy & Payen, 1988). Juvenile hormones are terpenoid hormones (Downer & Laufer, 1983). Three different forms, JH-I, JH-II, JH-III, are present in arthropods.

Implants of mandibular organ can stimulate vitellogenesis in the crab, Callinectes sapidus and Libinia emarginata (Hinsch, 1980, cited in Quackenbush, 1986; Yudin et al., 1980). Laufer et al. (1984) reported that isolated mandibular organ from the spider crab, Libinia emarginata, produced a JH-like compound. Methylfarnesoate, a JH-like compound, is a major product in the secretion of mandibular organ (Laufer et al., 1986). After eyestalk ablation, the secretion of MF was enhanced by at least 2 folds. The highest rate of MF in female was observed near the end of ovarian cycle. The authors postulated a role of juvenoids in vitellogenesis, most likely mediated by the stimulation of vitellogenin synthesis. The effect of MF on

reproduction may be causative since mandibular organ implants stimulate ovarian development in non-reproductive female L. emarginata (Hinsch, 1980). Up to now, there is still little information to confirm the relationship between the juvenoids and vitellogenesis.

2.2.1.2.5 Pheromones

Crustaceans may release pheromones into the water to attract a mate. In the freshwater shrimp, Paratya compressa, vitellogenesis is delayed when females are reared in the absence of males (Takayanagi et al., 1986). An extract of the testis or the vas deferens can resume ovarian maturation. Thus, Takayanagi suggested that organs of mature male shrimps, particularly the testis and vas deferens, secrete an ovary-stimulating pheromone which accelerates ovarian development.

2.2.1.2.6 Vertebrate-like steroids

A number of sex steroids, including testosterone, progesterone and pregnenolone have been identified in the gonads and serum of lobsters and crayfish (Burns *et al.*, 1984; Ollevier et al., 1986). Of these, progesterone may be of particular interest. Injections of progesterone and 17-α-hydroxyprogesterone have been reported to stimulate vitellogenesis in penaeid shrimp (Yano, 1985, 1987). Couch

et al. (1987) found estradiol in a number of lobster tissues, with the highest levels observed in the mandibular organ. Progesterone levels in immature animals were low or undetectable in all tissues tested except the mandibular organ. Further studies would be necessary to put forward a hypothesis on the role of vertebrate-like hormones in Crustacea.

2.2.2 Environmental factors

Reproduction is vastly seasonal or cyclic in crustaceans as in other animals. Environmental parameters such as temperature, photoperiod and salinity, may influence the initiation of puberty and reproductive behaviour. Among the factors, photoperiod and temperature were investigated to a large extent.

Manipulation of environmental conditions has been used successfully to induce ovarian maturation of several species of decapod crustaceans. Laubier-Bonichon (1978; cited in Crocos & Kerr, 1986) found that unablated Penaeus japonicus showed better maturation and spawning response under a 26°C, 16 h daylength regime than a 24°C, 14 h daylength regime, with no maturation occurring under conditions of 20°C and 12 h daylength. Brown et al. (1980) found that both ablated and unablated female P. stylirostris matured and spawned under conditions of 29°C and a 14L:10D photoperiod regime. Crocos and Kerr (1986)

reported that a combination of raised temperature (26°C), increased daylength (14.5 h) was sufficient to induce maturation and spawning of unablated P. esculentus. Control of photoperiod and temperature to stimulate ovarian maturation is still in the stage of trial-and-error.

Nelson (1986) discussed the relationship between photoperiod and reproduction in the american lobsters, Homarus americanus. Seasonal difference of photoperiod and water temperature in the habitats was believed to govern the reproduction of the lobsters. Inshore lobsters take both photoperiod and a drastic yearly fluctuation in water temperature as the seasonal cues. Their peculiar life history dictates that they spawn their eggs at a time which enables their larvae to take maximal advantage of summer conditions, i.e. long photoperiod and warm seawater. This reasoning may also apply to other decapods, such as freshwater crayfish, Orconectes virilis (Aiken, 1969) and blue crab Callinectes sapidus (Oesterling & Provenzano, 1985). Ovarian maturation of O. virilis occurs in spring, with long photoperiod and warm water. Mating and ovarian maturation of Callinectes sapidus take place in estuaries during the spring. In more northern area of the distribution of H. americanus, temperature is the determinant factor of ovarian maturation. At temperatures below 5°C during the long winter, embryonic development becomes very slow, moulting ceases, and perhaps vitellogenesis is also not possible. A rapid vitellogenic

response to temperature increase during the short summer, from April to July, may be essential if the species is to survive in these areas. On the other hand, offshore lobsters would find temperature to be an unreliable cue. There is no clear annual cycle of temperature change in deep water. Temperature fluctuation seems to depend on vagaries of offshore current pattern. Thus, the lobster may have come to rely on photoperiod to time vitellogenesis. Ovarian maturation of offshore lobster occurs during the long photoperiod in the summer. The photoperiodic response of ovarian maturation is species dependent. The American lobster, Homarus americanus displays a two phase vitellogenic response in the laboratory (Nelson, 1986). Short days are necessary to sustain early vitellogenesis, long day to trigger secondary vitellogenesis. Absence of response of ovarian maturation to photoperiod is found in the European lobster, H. gammarus (Nelson, 1986).

Photoperiod and temperature may influence ovarian maturation of decapod crustaceans through the X organ-sinus gland complex of the eyestalk via gonad inhibiting hormone (GIH), perhaps together with some other neurohormones. The relationship between the receptors of environmental factors and the neurosecretory system is obscure.

3.2.3 Nutritional factors

In many insects, deprivation of food does not alter sexual behaviour and fertilizing capacity (Adiyodi & Adiyodi, 1974). Yet in many other arthropods including crustaceans, food is essential for egg maturation and normal oviposition. Food provides not only the raw materials and essential nutritive factors for yolk synthesis and accessory sex secretions, but also serves, mixed or unmixed with mating and other stimuli, to gear the appropriate neural and hormonal machinery. Therefore, a suitable diet is a critical factor in ovarian maturation.

Reliable data on the nutrient requirement specific to maturation, reproduction and embryogenesis in crustaceans are scant and fragmentary. In commercial hatcheries, mollusks including squid, mussel and clam are commonly fed to shrimp broodstock (Primavera, 1988). In America, the marine polychaete, Glycera dibranchiata, has been used by shrimp farmers as a reliable food source to stimulate the maturation for a long time (Lytle et al., 1990). Some degrees of successful spawning of the shrimp, Penaeus vannamei fed squid along with one or more mollusks, such as mussels, clams or oysters in addition to marine polychaetes have been reported (Ogle, 1988, cited in Lytle et al., 1990). In wild adult Penaeus monodon, 85% of ingested food of consists of mollusks, small crabs and shrimps (Matre, 1980). The more frequent occurrence of mollusks, polychaetes and other non-crustaceans in the gut is found

during the spawning season (Matre, 1982). Thus, some biochemical ingredients in the polychaetes and mollusks may be necessary for reproduction of decapod crustaceans.

Middleditch et al. (1979, 1980a,b) were the first to investigate the importance of a maturation diet in penaeid shrimp. In these studies, high content of polyunsaturated fatty acids and cholesterol were found in marine polychaetes and bivalves (Middleditch et al., 1980b). In studies of fatty acids in the diet of penaeid shrimp, Middleditch et al. (1980a) established that a diet with supplemental enrichment of polyunsaturated fatty acids (PUFA) was needed for maturation. The best dietary fatty acids supplement was found to be the Maine bloodworm, Glycera dibranchiata. Analysis of the fatty acid composition of this polychaete shows that it contains plenty of polyunsaturated essential fatty acids, the ω6, or linoleic family (18:2ω6), and the ω3, or linolenic family (18:3ω3). These two families of polyunsaturated fatty acids seem to affect crustacean reproduction. A certain balance of these two essential fatty acid families in the diet appears to play an important role in hormonal functions (Castell, 1982). Uptake of cholesterol in the ovary during maturation of ablated shrimp, Penaeus japonicus indicates that cholesterol is an essential nutrient for reproduction in crustaceans (Kanazawa et al., 1988).

Besides analysis of the diets, information on the nutrient requirements of shrimp broodstock can also be deduced from the changes in biochemical compositions of the organs, such as the gonads and hepatopancreas, and the eggs. Details will be discussed in the following section.

2.3 The role of lipids during ovarian maturation of decapod crustaceans

2.3.1 Lipids in decapod crustaceans

Lipids are a large and diverse group of organic compounds which include triglycerides, phospholipids, cholesterol and fatty acids. Lipids serve not only as vital energy stores and crucial constituents of cellular and subcellular membranes, but they also appear to play regulatory roles as hormones.

Basically, lipids usually in the form of triglycerides provide a pool of energy source, and are the predominant form of energy storage in the adult, egg and pre-feeding larva of crustaceans (Middleditch et al., 1979; Ward et al., 1979; Clarke, 1982; Teshima & Kanazawa, 1983). Hydrolysis of the ester linkage of triglycerides will liberate glycerol and three fatty acid molecules. Under ß-oxidation in the mitochondria, acetyl groups will be released and combine with coenzyme A to form acetyl Co A which then enters the tricarboxylic acid (TCA) cycle to liberate ATP. Lipids such as triglycerides will give twice the energy of protein and carbohydrate on a per weight basis. Thus many animals, including crustaceans, store triglycerides as an energy reserve.

Phospholipids (polar lipids) are another abundant lipid class in crustaceans. They have an essential

structural function in cellular membranes, affecting structural and physiological properties. Moreover, phospholipids are important for transport of triglycerides or other neutral lipids, such as cholesterol, in the haemolymph of crustaceans (D'Abramo and Lovell, 1991). This is different from insects where triglycerides are transported by diglycerides (Gilbert & O'Connor, 1970). In crustaceans, triglycerides are transformed to phospholipids in the hepatopancreas after absorption. They then combine with protein to form lipoproteins which are transported to other parts of body.

Cholesterol is another major and essential component of lipids in decapod crustaceans. Unlike other animals such as vertebrates, the crustaceans are incapable of synthesize sterols de novo (Zandee, 1967). They cannot synthesize cholesterol from either acetate or mevalonate. In studies using ^{14}C-acetate, no incorporation into sterols of the shrimp Penaeus japonicus, the spiny lobster Panulirus japonicus and the crab Portunus trituberculatus was found, although fatty acids were labelled (Teshima & Kanazawa et al., 1971). Therefore, decapod crustaceans rely on the dietary cholesterol for growth and survival. The necessity of dietary cholesterol for good growth was demonstrated in many decapods, such as the shrimp Penaeus japonicus (Kanazawa et al., 1971; Deshimaru & Kuroki, 1974), the lobster Homarus americanus (Castell et al., 1975; D'Abramo et al., 1984), and the crayfish Pacifastacus

leniusculus (D'Abramo et al., 1985). Abundant cholesterol is necessary for maintenance of membranous structure. Some decapods, such as the shrimps, Penaeus japonicus, and Palaemon serratus, the lobster, Homarus americanus, and the crabs, such as Portunus trituberculatus and Sesarma dehaani, are able to convert dietary cholesterol to steroid hormones, such as progesterone, 17-hydroxyprogesterone, androstenedione, testosterone and ecdysone (Teshima, 1982, review).

Fatty acids are one of the main components of lipids. They appear not only in the form of free fatty acids, but also as a moiety of other lipid classes, such as triglycerides, phospholipids and wax esters. Fatty acids can be divided into three major groups: saturated, monounsaturated and polyunsaturated fatty acids. In general, saturated and monounsaturated fatty acids are always incorporated into neutral lipids, such as triglycerides and wax esters, while polyunsaturated fatty acids (PUFA) are always incorporated in polar lipids, particularly phospholipids.

Decapods usually contain high concentrations of palmitic (16:0), stearic (18:0), palmitoleic (16:1), oleic (18:1), arachidonic (20:4ω6), 5,8,11,14,17-eicosapentaenoic (20:5ω3), and 4,7,10,13,16,19-docosahexaenoic (22:6ω3) acids (Guary et al., 1974; Teshima et al., 1976; Clarke, 1977, 1979, 1982; Middleditch et al., 1980a; Teshima & Kanazawa, 1983; Jeckel et al., 1989).

Crustaceans are capable of de novo synthesis, in an efficient manner, of palmitic acid (16:0) and of incorporating it into its acylglycerols, as well as converting it to 16:1ω7 by δ9 desaturation and to 18:0 and 18:1ω9 fatty acids by elongation and desaturation reaction (Moreno et al., 1979a,b). However, unlike vertebrates, crustaceans are unable to elongate long unsaturated fatty acids or introduce a second or third double bond into long fatty acids. Thus, crustaceans are exclusively dependent upon exogenous sources on the polyunsaturated fatty acids (PUFA) with 20 and 22 carbon. The dietary requirement of these acids may be specific to the families of fatty acids (D'Abramo & Lovell, 1991). Guary et al. (1976) and Kanazawa et al. (1977) found that Penaeus japonicus grows more rapidly if the PUFA source is 18:3ω3 rather than 18:2ω6.

Salinity may be a factor affecting fatty acid compositions among decapod crustaceans. The linoleic acid (ω6) series is dominant in freshwater crustaceans while the linolenic acid (ω3) series predominates in marine crustaceans (Castell, 1982). Large amounts of ω3-type of polyunsaturated fatty acids have been reported in the shrimp Penaeus japonicus (Teshima *et al.*, 1976; Guary et al., 1974), the queen crab Chionectes opilio (Addision et al., 1972), and the crab Helice tridens (Teshima et al., 1976). The marine species also have higher proportions of longer chain (20 and 22 carbon) fatty acids than freshwater

species. Among polyunsaturated fatty acids, arachidonic acid (20:4ω6), eicosapentaenoic (20:5ω3) and docosahexaenoic (22:6ω3) acids predominate in marine species. The freshwater shrimp Palaemon paucidens contains higher percentage of arachidonic acid (20:4ω6) and lower percentage of linoleic acid (18:2ω6) than marine crustaceans (Teshima et al., 1976). Similar results were reported in the crayfish Orconectes rusticus (Wolfe et al., 1965, cited in Teshima et al., 1976) and O. virilis (O'Connor & Gilbert, 1968).

The pattern for saturated and monounsaturated fatty acids appears to vary considerably between species and does not show any apparent relationship with salinity (Castell, 1982). Teshima et al. (1976) showed that in marine decapod palmitic (16:0) and oleic (18:1) acids constitute about half of the saturated and monounsaturated acid fractions respectively, and freshwater decapods contain more palmitoleic (16:1) and oleic (18:1) acids, but less stearic acid (18:0) than marine species.

2.3.2 Variation of lipids during ovarian maturation

2.3.2.1 Introduction

Changes in biochemical constituents are pronounced in invertebrates which exhibit reproductive cycle, since a

great amount of energy must be channelled to the gonads during reproduction. This phenomenon reflects in the deposition or depletion of nutrients in different tissues during reproduction. The predominant organic reserves of many crustaceans are glycogen and lipids (O'Connor & Gilbert, 1968) which are accumulated in the hepatopancreas and ovary during gonad development. Moreover, the embryo and pre-feeding larvae of crustaceans are lecithotropic, which means that the energy demand during these periods is solely supplied by egg yolk reserves (Harrison, 1991). The quality and quantity of nutrients in eggs yolk are dependent on maternal reserves, the capacity of biosynthesis, and dietary intake during ovarian maturation.

The ovary of crustaceans is shown to contain higher level of lipids than other organs (Morris, 1973; Guary, et al., 1974). Lipid deposition in the ovary during ovarian maturation has been demonstrated by microscopy and histochemistry in a number of crustaceans, including the crayfish Orconestes sp., Astacus fluviatilis, the shrimps Crangon crangon, Penaeus monodon, and several species of Palaemonetes (Beams & Kessel, 1963; Brodzicki, 1963).

Fatty acids in eggs of decapods are relatively rich in monounsaturated acids compared with those in adult decapods. The proportion of monounsaturated fatty acids, such as palmitoleic (16:1), oleic (18:1), and eicosenoic (20:1) acids, are relatively higher in the eggs (Clarke, 1979; Lautier & Lagarrigue, 1988). This findings may be

related to the increase in the proportion of monounsaturated fatty acids in the ovary of maturing females. Polyunsaturated fatty acids in the eggs make up a larger portion of total fatty acids than in the ovary. For instance, polyunsaturated fatty acids compose about 30% of total fatty acids in the eggs of the crab Pachygrapsus marmoratus (Lautier & Lagarrigue, 1988). The relative high amount of polyunsaturated fatty acids is reflected in high percentage of ω3-type fatty acids, particularly linolenic (18:3), eicosapentaenoic (20:5) and docosahexaenoic acids (22:6).

Polyunsaturated fatty acids are essential for marine fish and crustaceans (Middleditch et al., 1980a). Prostaglandins which are involved in the control of many physiological processes are derived from polyunsaturated fatty acids. The hormonal effect of prostaglandins in mammalian reproductive system is well documented (Jones, 1987; Xavier, 1987). Three common acids, arachidonic, eicosapentaenoic and docosahexaenoic acids, have been shown to be important in fish reproduction (Moore, 1987). Thus polyunsaturated fatty acids may also be a crucial factor in the regulation of crustacean reproduction.

In the following sections, the changes of total lipids, lipid classes and fatty acid compositions in the ovary and hepatopancreas during ovarian maturation of decapod crustaceans will be discussed.

2.3.2.2 Changes of lipids in the ovary

Changes in biochemical composition of the ovary during maturation have been reported in some crustaceans. Pillay and Nair (1973) correlated increases in percentages of both lipid and protein in the ovaries with the increases in the gonad indices from minimal reproductive activity to peak activity in the shrimp, Metapenaeus affinis, and the crabs, Portunus pelagicus and Uca annulipes. Using the criteria of Cummings (1961) for the division of maturation stages, Gehring (1974) reported that in Penaeus duorarum, the total lipids in the ovary increased between the developing and nearly ripe stages. However, there was a decrease in the total lipid of the ovaries between the nearly ripe and ripe stages.

Teshima & Kanazawa (1983) demonstrated a similar pattern of change in ovarian lipid of Penaeus japonicus. The ovarian maturation is divided to five stages using the gonadosomatic index as the criteria. The ovarian lipid concentration increased during the developing and slightly mature stages. The concentration remained at high and roughly constant levels during the mature stage to spawning, and then decreased to low levels in spent ovaries. The absolute amount of lipid accumulated in the ovary increased from 50 mg to 1300 mg per shrimp along ovarian maturation.

A tremendous increase of lipid in the ovary is also documented in other decapods, such as in the penaeid shrimps Parapenaeopsis hardwickii (Kulkarni & Nagabhushanam, 1979), Penaeus indicus (Galois, 1984), P. setiferus , P. aztecus (Castille & Lawrence, 1989), P. monodon (Millamena & Pascual, 1990), and P. kerathurus (Mourente & Rodriguez, 1991), as well as the crabs Paratelphusa hydrodromous (Adiyodi & Adiyodi, 1971), Cancer magister (Allen, 1972), Carcinus maenas (Paulus & Laufer, 1982), and Pachygrapsus marmoratus (Lautier & Lagarrigue, 1988).

Part of the lipids is used in the ovarian tissue for the manufacture of oocytes and the rest is shunted into the oocytes for future uses as energy production and cellular constituents. The amount of egg lipids was found to be 11% frozen weight in the shrimp Pandalus montagui (Clarke, 1979) and 38% dry weight in the crab Pachygrapsus marmoratus (Lautier & Lagarrigue, 1988).

Lipids in crustacean oocytes and spawned eggs are stored in two ways, either as glycolipoproteins, rich in polar lipids, or as fat droplets mainly in the form of triglycerides (Wallace et al., 1967; Zagalsky et al., 1967; Fyffe & O'Connor, 1974). Lautier and Lagarrigue (1988) showed that triglycerides and phospholipids are the dominant lipid classes in the eggs of the crab, Pachygrapsus marmoratus. The amount of triglycerides and

phospholipids were 40% and 43% of total lipids, respectively.

Cholesterol and triglycerides are the two major neutral lipid fractions, comprising more than 90% of neutral lipids in all stages, in the ovary of Penaeus duorarum (Gehring, 1974), P. kerathurus (Mourente & Rodriguez, 1991) and the crab, Pachygrapsus marmoratus (Lautier & Lagarrigue, 1988). They both increased during ovarian maturation but cholesterol increased less significantly than triglycerides. In the shrimp, Penaeus kerathurus, the concentration of ovarian cholesterol increased from 0.3% of tissue dry weight in immature stage to 1.3% in mature stage (Mourente & Rodriguez, 1991). During the same period, the concentration of triglycerides increased from 1.4% to 5.9%. In the crab, Pachygrapsus marmoratus, the concentrations of cholesterol and triglycerides increased from 4.7% and 13.3% at immature stage to 6.2% and 15.2% at mature stage, respectively. The ovary is presumably the major site of cholesterol metabolism, followed by the hepatopancreas (Kanazawa et al., 1988), the cholesterol accumulated may be incorporated in the oocytes or transformed to other sterols.

The concentration of phospholipids in the ovary of Penaeus kerathurus increases from 7.9% of tissue dry weight at immature stage to 12.5% at mature stage (Mourente & Rodriguez, 1991). In the crab, Pachygrapsus marmoratus, the concentration of ovarian phospholipids increased from

15.5% of tissue dry weight at immature stage to 20.0% at mature stage (Lautier & Lagarrigue, 1988). The simultaneous increases of phospholipids and proteins during ovarian maturation of the shrimps Penaeus aztecus, P. setiferus (Castille & Lawrence, 1989) and Pleoticus muelleri (Jeckel et al., 1989) suggest that the largest part of these components exist as lipoproteins or glycolipoproteins together with substantial quantities of neutral lipids, especially triglycerides. These substances may be stored in the form of yolk in the oocytes. Phosphatidylcholine and phosphatidylethanolamine are the dominant classes of ovarian polar lipids. They were responsible for the changes in ovarian polar lipids in the shrimps Pandalus montagui (Clarke, 1979), Penaeus duorarum (Gehring, 1974), P. kerathurus (Mourente & Rodriguez, 1991), and Pleoticus muelleri (Jeckel et al., 1989).

The profile of fatty acids present in the mature ovary is a reflection of the fatty acid requirement of that tissue or the request for the developing embryos after fertilization (Millamena & Pascual, 1990). The requirement of such acids in the ovary may be the determining factor for lipid storage and conversion in the hepatopancreas. Generally, the major fatty acids in the ovary are palmitic (16:0), palmitoleic (16:1), stearic (18:0), arachidonic (20:4ω6), eicosapentaenoic (20:5ω3) and docosahexaenoic (22:6ω3) acids (Guary et al., 1974; Teshima et al., 1976;

Clarke, 1977, 1979, 1982; Middleditch et al., 1980a; Teshima & Kanazawa, 1983; Jeckel et al., 1989).

During ovarian maturation with an accumulation of triglycerides, the saturated fatty acids increase at the expense of polyunsaturated fatty acids. Palmitic (16:0) and oleic (18:0) acids are mainly responsible for the increase in fatty acid concentration in the shrimp Penaeus kerathurus (Mourente & Rodriguez, 1991). Similar results were obtained in the shrimps Penaeus japonicus (Guary et al., 1974) and Pleoticus muelleri (Jeckel et al., 1989), and the crab Cancer magister (Allen, 1972). In the crab Pachygrapsus marmoratus, the concentration of saturated fatty acids remains roughly constant while the concentration of monounsaturated and polyunsaturated fatty acids increase during ovarian maturation (Lautier & Lagarrigue, 1988).

Similar to the results in the eggs, the crustacean ovary generally contain high levels of monounsaturated acids (Morris, 1973). The proportion of palmitoleic acid (16:1) is higher in lipids of the ovary and hepatopancreas than in the lipid of haemolymph and muscle throughout the process of ovarian development (Allen, 1972; Teshima & Kanazawa, 1983; Jeckel et al., 1989). Guary et al. (1974) demonstrated the presence of high levels of monounsaturated fatty acids in the ovary of Penaeus japonicus and postulated the necessity of these fatty acids for building up the triglycerides during ovarian maturation.

The predominant polyunsaturated fatty acids are arachidonic (20:4ω6), eicosapentaenoic (20:5ω3) and docosahexaenoic (22:6ω3) acids in the mature ovary of the shrimps, Penaeus japonicus (Teshima & Kanazawa, 1983), P. monodon (Millamena & Pascual, 1990), and P. kerathurus (Mourente & Rodriguez, 1991). Generally, higher proportions of total polyunsaturated fatty acids are found in polar lipids fraction than in the neutral lipids fractions (Millamena & Pascual, 1990; Mourente & Rodriguez, 1991). It is found that in the shrimps Penaeus monodon and P. kerathurus the increase in the concentrations of these polyunsaturated fatty acids, 20:4ω6, 20:5ω3 and 22:6ω3, in the polar lipid fraction of mature ovary coincides with the decrease in concentrations of these acids in the hepatopancreas at that stage (Millamena & Pascual, 1990; Mourente & Rodriguez, 1991). This phenomenon further indicates the importance of polyunsaturated fatty acids in ovarian development. A depletion in polyunsaturated fatty acids of the polar lipids at mature stage suggests the possibility of conversion of the acids into other compounds for incorporation into the eggs or their use as an energy source.

2.3.2.2 Changes of lipids in the hepatopancreas

The crustacean hepatopancreas is generally regarded as a major lipid storage organ, analogous to the fat body in

insects and adipose tissue and liver in vertebrates (Huggins & Munday, 1968). Dall (1981) stated that the major role of lipid stored in hepatopancreas is to act as energy reserve for vitellogenesis and moulting. Owing to the high lipid requirement for ovarian maturation, the hepatopancreas is regarded as a metabolic nucleus converting incoming lipids to yolk components rather than simply as a reservoir of source of yolk (Clarke, 1982; Harrison, 1991).

The concentrations of hepatopancreatic lipid increase with increasing maturity of the ovaries in Penaeus japonicus, reaching a maximum level at the slightly mature stage of ovaries, and then decreased during the mature stage to spawning (Teshima & Kanazawa, 1983). The decrease in the lipid concentration of the hepatopancreas with a concurrent increase in ovarian lipids was also found during ovarian maturation. These results suggested the possible movement of hepatopancreatic lipids to the ovaries during ovarian maturation. This process calls for a mobilization of energy reserves to provide nutrients for the eggs in the ovary.

Mobilization of hepatopancreatic lipid to the ovary has been reported in other crustaceans such as other penaeids, including Parapenaeopsis hardwickii (Kulkarni & Nagabhushanam, 1979), Penaeus indicus (Galois, 1984), P. kerathurus (Mourente & Rodriguez, 1991), P. monodon (Millamena & Pascual, 1990), and P. setiferus (Castille &

Lawrence, 1989) and the crabs Paratelphusa hydrodromous (Adiyodi & Adiyodi, 1971), Cancer magister (Allen, 1972), Carcinus maenas (Paulus & Laufer, 1982) and Pachygrapsus marmoratus (Lautier & Lagarrigue, 1988). The mobilization during ovarian maturation was confirmed by a large increase of total lipid in the haemolymph in the shrimp Penaeus japonicus (Teshima & Kanazawa, 1983) and the crab Pachygrapsus marmoratus (Lautier & Lagarrigue, 1988).

Phospholipids and triglycerides are the major components of hepatopancreatic lipids. These two classes are responsible for the changes in hepatopancreatic lipid during ovarian maturation. Their concentrations always remain high during the earlier maturation stages, and then decrease at the subsequent stages until spawning. In the crab Pachygrapsus marmoratus, the concentrations of phospholipids and triglycerides decreased from 19.1% and 14.8% of tissue dry weight at immature stage to 8.6% and 6.8% at mature stage, respectively (Lautier & Lagarrigue, 1988). Similar results were also observed in penaeids, such as Penaeus japonicus (Teshima & Kanazawa, 1983) P. monodon (Millamena & Pascual, 1990) and P. kerathurus (Mourente & Rodriguez, 1991). These studies also showed that the concentrations of neutral lipids in hepatopancreas decrease during the later stages of ovarian maturation. This decrease was mainly attributed to a decrease in triglycerides. On the other hand, phosphatidylcholine and phosphatidylethanolamine were dominant in polar lipids, and

responsible for the changes of polar lipids in the hepatopancreas.

During digestion, ingested triglycerides are enzymatically cleaved, mainly by triglyceride lipases, to either 1,2-diglycerides and 2-monoglycerides. Both glycerides are probably resynthesized to triglycerides or converted to phospholipids through absorptive cells of the hepatopancreas (Chang & O'Connor, 1983, Harrison, 1990). Afterwards, triglycerides in the hepatopancreas can be transported to ovaries as lipoproteins rich in phospholipids during gonad maturation (Teshima & Kanazawa, 1983).

During ovarian maturation, concentration of cholesterol in the hepatopancreas decreased at the later maturation stages in the shrimps Penaeus japonicus (Teshima & Kanazawa, 1983) and P. indicus (Galois, 1984), and the crab Pachygrapsus marmoratus (Lautier & Lagarrigue, 1988). During vitellogenesis, dietary esterified sterols were converted into free sterol in the hepatopancreas. Then these free sterols were transported to ovary for oocyte formation. Thus, the hepatopancreas seems to be the principal site of cholesterol absorption and storage (Mourente & Rodriguez, 1991).

The major hepatopancreatic fatty acids are palmitic (16:0), stearic (18:0), palmitoleic (16:1), oleic (18:1), eicosenoic (20:1), arachidonic (20:4ω6), eicosapentaenoic (20:5ω3) and docosahexaenoic (22:6ω3) acids (Guary et al.,

1974; Teshima et al., 1976; Clarke, 1977, 1979, 1982; Middleditch et al., 1980a; Teshima & Kanazawa, 1983; Jeckel et al., 1989). The concentrations of all hepatopancreatic fatty acids increase by the end of developing stage of Penaeus monodon (Millamena & Pascual, 1990). Major changes occur in palmitoleic (16:1), eicosapentaenoic (20:5ω3) and docosahexaenoic (22:6ω3) acids. A noteworthy decline of total fatty acid concentrations occurs during the subsequent stages. This result corresponds with the marked increase in total fatty acid contents in the ovary during the same period. Similar results were reported in the crabs, Paratelphusa hydrodromous (Adiyodi & Adiyodi, 1971) Pachygrapsus marmoratus (Lautier & Lagarrigue, 1988), Penaeus japonicus (Teshima & Kanazawa, 1983) and the shrimp, P. kerathurus (Mourente & Rodriguez, 1991). The decline of total fatty acid concentrations is mainly due to the saturated and monounsaturated fatty acids. During the mature stages, there is still a progressive increase in polyunsaturated fatty acids. This result suggests that the hepatopancreas is a reservoir for these acids.

Chapter 3 Variation of lipid composition during ovarian maturation of Penaeus chinenesis

3.1 Introduction

This chapter presents the variation of lipids composition in ovary, hepatopancreas and muscle during ovarian maturation in Penaeus chinensis.

3.2 Materials and methods

3.2.1 Experimental animals

Wild adult female Penaeus chinensis were acquired from the fish market at Tuen Mun. Females were transported alive to the laboratory. Shrimps at different stages of ovarian maturation were separated. Five stages could be identified, based on a scheme modified from Yano (1985).

Stage I (Immature stage):

The ovary was translucent, unpigmented with no distinguishable outline.

Stage II (Developing stage):

The ovary was visible as a thick opaque line along the dorsal central axis.

Stage III (Slightly mature):

The ovary was visible through the exoskeleton and yellow in colour.

Stage IV (Mature stage):

The ovarian lobes were larger than in the preceding stage and clearly visible through the exoskeleton. The colour of ovary changed to green.

Stage V (Spent ovary stage):

Shrimp just after spawning were used.

After the wet weight and carapace length of the shrimp were measured, the ovaries, hepatopancreas and a portion of abdominal muscle were dissected out. Carapace length was measured from the posterior orbital margin to the posterior edge in the mid-dorsal line. The samples and the rest of the body were immediately frozen in liquid nitrogen, and freeze-dried for a week. The samples were weighed and the gonadosomatic (GSI = weight of ovary x 100/ body weight) and hepatosomatic (HSI = weight of hepatopancreas x 100/ body weight) indices were calculated. The samples were stored at -70°C until analysis.

3.2.2 Total lipid extraction and quantification

Total lipids were extracted according to the method of Sasaki and Capuzzo (1984) and lipid contents were determined by the method of Barnes and Blackstock (1973). The detailed experimental procedures were presented below.

3.2.2.1 Total lipid extraction

About 400 mg tissue was placed into a homogenizing flask. After homogenizing in 5-ml distilled water, 7 ml of chloroform-methanol (1:2, v/v) solution was added. The mixture was homogenized for 3 minutes. The solution was transferred into a centrifuge tube. After centrifuged at 52000 x g for 10 minutes, the chloroform layer was pipetted out and saved. Another 7 ml of chloroform-methanol solution (1:1, v/v) was introduced into the centrifuge tube. The mixture was homogenized for 3 minutes. After centrifuged at 52000 x g for 10 minutes, the chloroform layer was pipetted out and saved. 4 ml of chloroform-methanol (3:1, v/v) was then added in the centrifuge tube. The whole solution was homogenized for 3 minutes. After centrifuged at 52000 x g for 10 minutes, the third portion of chloroform layer was pipetted out and saved. All three portions of chloroform layer were pooled into another centrifuge tube and 5 ml of 0.7% sodium chloride solution was added. The mixture was homogenized for 10 minutes. After centrifuged at 52000 x g for 20 minutes, the chloroform layer was pipetted out for further analysis.

3.2.2.2 Quantification of total lipid content

Aliquots of lipid extract (0.5 ml equivalent to 5 mg dry tissue each) were transferred to 150 x 16 mm Pyrex test

tubes for analysis. Each batch of analysis also included 0.5 ml of chloroform as the blank.

All aliquots were dried by nitrogen and 0.5 ml concentrated sulphuric acid was added to the residue. After mixing, the solution was heated for 10 minutes in a boiling water bath. 0.1 ml aliquots of the acid digest was transferred to clean dry test tubes and 2.5 ml of vanillin reagent was added. The solution was mixed thoroughly and allowed to stand at room temperature for 30 minutes. Finally, the absorbance of each solution was recorded at 520 nm. The amount of lipids in the solution was estimated by a calibration curve obtained with a series of respective standard solutions of cholesterol.

3.2.3 Separation and quantification of lipid classes

Lipid classes were separated with one-dimensional, ascending thin layer chromatography (TLC). The chloroform extracts were concentrated by nitrogen and spotted on TLC plates coated with silica gel (Merck Cat. No. 5714).

3.2.3.1 Separation of total lipid classes

Lipid extracts (10-50 μl equivalent to 0.4 to 2 mg of dry tissue) were applied on TLC plates. The plate was developed in isopropyl ether/acetic acid (96:4, v/v) at room temperature (Skipski & Barclay, 1969). The solvent

was allowed to ascend for 8 cm and the plate was air-dried at room temperature for 45 minutes. Neutral and polar lipids were separated. To separate different neutral lipids, the plate was then developed in the same direction with petroleum ether/diethyl ether/acetic acid (90:10:1, v/v/v) until the solvent front was within 1 cm of the top of the plate. The plate was air-dried and the spots were visualized by iodine vapour. Individual standards and standard mixtures were spotted alongside lipid samples to compare R_f values. The neutral lipids separated included monoglycerides, diglycerides, triglycerides, cholesterol and free fatty acids. The total neutral lipids was calculated as the sum of all neutral lipid classes.

3.2.3.2 Separation of polar lipid classes

After application of samples on TLC plate, the plate was developed in acetone/petroleum ether (1:3, v/v) at room temperature (Skipski & Barclay, 1969). The solvent was allowed to migrate to top of the plate and the plate was air-dried at room temperature for 45 minutes. The plate was then developed, in the same direction with chloroform/methanol/acetic acid/water (25:15:4:2, v/v) until the solvent front was within 2.0 cm of the top of the plate. The plate was air-dried and the spots were visualized by iodine vapour. Individual standards and standard mixtures were spotted alongside lipid samples to

compare R_f values. Sphingomyelin, phosphatidylcholine, phosphatidylserine, and phosphatidylethanolamine were separated.

3.2.3.3 Quantification of lipid classes

The concentrations of lipid classes, including classes of polar lipids, were determined with the dichromate-oxidizing method (Amenta, 1964). After scraping off the silica gel of the particular spots, the lipids were extracted with chloroform. The chloroform extracts were then evaporated to dryness by nitrogen. Acidic potassium dichromate solution (1.5 ml) was added. The whole mixture was heated in boiling water bath for 20 minutes. After cooling, 1.0 ml solution was pipetted out to another test tube. The solution was diluted by 10-ml distilled water. After mixing thoroughly, the absorbance of each solution was recorded at 350 nm, using water as a blank. The amount of lipids in the solution was estimated by a calibration curve obtained with a series of respective standard compounds.

3.2.4 Fatty acid analysis

3.2.4.1 Preparation of fatty acid methyl esters (FAME)

The concentration of fatty acids in total lipids, neutral lipids and polar lipids were determined. Neutral lipids and polar lipids were obtained after separation of total lipids with TLC developed in isopropyl ether/acetic acid (96:4, v/v) (see 3.2.3.1). Lipids were extracted then with chloroform.

Fatty acid methyl esters were prepared from lipid extracts based on Metcalf *et al.* (1966) and van Wijngaarden (1967) with modifications by Capuzzo & Sasaki (1984). The chloroform extract was placed in a 50-ml round bottom flask and evaporated to dryness with a stream of nitrogen at 40°C. A solution of 8.0 ml of 0.5 M sodium hydroxide in methanol was then added. The solution was refluxed in boiling water bath for 1.5 h. After cooling, 14% boron trifluoride-methanol mixture was added. The solution was further refluxed for 30 minutes. The solution was cooled and transferred to a centrifuge tube. 3 ml pentane was added and the whole solution was mixed thoroughly. 4 ml of saturated sodium chloride solution in water was then added and the whole solution was centrifuged at 60000 x g for 5 minutes. The upper pentane layer was pipetted out and saved. Another portion of 3 ml pentane was added. After centrifugation, the pentane was collected and pooled with the first fraction. The combined pentane extract was

evaporated to dryness with nitrogen. During analysis, the extracts were redissolved in HPLC-grade hexane and methyl heneicosanoate was added to act as an internal standard.

3.1.4.2 Gas chromatography

The fatty acid methyl esters were analyzed with a Varian 3400 gas chromatograph equipped with flame ionization detector and a fused silica capillary column (30 m x 0.32 mm i.d.) coated with 0.25 micron-thick Supelcowax-10 (polydiethylglycol, PEG) as the stationary phase (Supelco Inc., Bellefonte, USA). The temperature of the injector port and the detector were 200 and 250°C, respectively. Nitrogen was used as the carrier gas and the column temperature programme consisted of a temperature gradient from 150 to 190°C at a rate of 3°C/min. Individual methyl esters were identified by comparison with the relative retention times of known commercial standards (Supelco, Inc., Bellefonte, USA).

3.3 Results

3.3.1 Biometric data

The carapace length and body weight of Penaeus chinensis in each maturation stage are shown in Table 3.1. The size of the shrimps in term of these two parameters at different stages was similar. The gonadosomatic index (GSI) increased from 1.9% at stage I (immature stage) to 6.3% at stage IV, and then dropped to 2.0% at stage V (spent ovary stage). The hepatosomatic index (HSI) exhibited a slight decreasing trend along ovarian maturation but the differences are not statistically significant.

3.3.2 Variation of total lipids

The total lipid concentration of ovaries rose from 13% of tissue dry weight at stage I to 39% at stage IV, and the concentration decreased to 17% at stage V (Table 3.2). The hepatopancreatic lipid concentration appeared to be higher in stages I and II than in stages III and IV, but the differences are not statistically significant. The muscular lipid content, which comprised only about 5% of tissue dry weight, remained roughly the same during ovarian maturation.

It can be estimated that the absolute amount of ovarian lipid increased from 0.06 g per shrimp at stage I to 1.4 g per shrimp at stage IV, and then decreased to 0.08 g at stage V (Table 3.3). During the same period, the amount of hepatopancreatic lipid decreased from 0.79 g per shrimp at stage I to 0.47 g at stage V.

3.3.3 Variation of lipids in ovary

3.3.3.1 Neutral lipid classes

The neutral lipids accumulated in the ovary during ovarian maturation (Table 3.4). The concentration of total neutral lipids increased about three folds from stage I to IV, and dropped to a low level in spent ovaries. The predominant classes of ovarian neutral lipids were cholesterol, free fatty acids and triglycerides, which constituted more than 90% of neutral lipids. From stage I to IV, the concentrations of free fatty acids increased two folds while the concentration of cholesterol increased three folds. The concentration of triglycerides increased four folds but the variation is not statistically significant. The concentrations of these three components decreased in spent ovaries. Small amounts of diglycerides and monoglycerides of about 1 mg/g or lower were present and varied little among the stages.

3.3.3.2 Polar lipid classes

The concentration of polar lipids increased about two folds from stage I ti IV (Table 3.5). Phosphatidylcholine and phosphatidylethanolamine were the dominant polar lipid classes. These two components often constituted more than 50% of polar lipids. The concentration of phosphatidylcholine increased more than three folds from stage I to IV, and decreased to a low level in spent ovaries. The variation of other polar lipids are not statistically significant.

3.3.3.3 Fatty acid composition

Palmitic (16:0), palmitoleic (16:1) and oleic (18:1) acids, which constituted more than 60% of total fatty acids, were the major fatty acid components of ovarian lipids (Table 3.6b). Arachidonic (22:4ω6), eicosapentaenoic (20:5ω3) and docosahexaenoic (22:6ω3) acids were the major polyunsaturated fatty acids. The three acids constituted about 60% of the total polyunsaturated fatty acids.

The concentrations of saturated, monounsaturated, and polyunsaturated fatty acids in the ovary increased during ovarian maturation, and then decreased in spent ovaries (Table 3.6a). From stage I to IV, the concentrations of saturated and monounsaturated fatty acids increased 53% and

62%, respectively, and that of polyunsaturated fatty acids increased two folds. Their concentrations decreased to low levels in spent ovaries.

Similar fatty acid compositions and patterns of variation as in total lipids were found in ovarian polar and neutral lipids (Tables 3.7 and 3.8). The ovarian polar lipids contained higher proportions of polyunsaturated fatty acids, in particular eicosapentaenoic (20:5ω3) and docosahexaenoic (22:6ω3) acids, at the mature stage than those in earlier stages (Table 3.8b).

3.3.4 Variation of lipids in hepatopancreas

3.3.4.1 Neutral lipid classes

The concentration of total neutral lipids in the hepatopancreas increased about 30% from stage I to II (Table 3.9). The concentration then decreased in stage IV and was only half of the value at stage II. It increased about 30% in stage V. Triglycerides, cholesterol and free fatty acids were the major fractions in hepatopancreatic neutral lipids. These components often comprised more than 90% of neutral lipids at all stages. The concentrations of triglycerides and free fatty acids increased from 181 mg/g tissue dry weight and 92 mg/g at stage I to 211 mg/g and 123 mg/g at stage II, respectively. Their concentrations

then decreased to 102 mg/g and 34 mg/g at stage IV, respectively. In stage V, both triglycerides and free fatty acids increased to higher levels. The concentration of cholesterol increased about four folds from stage I to III, and appeared to be lower in stages IV and V. Small amounts of diglycerides and monoglycerides of less than 1.5 mg/g tissue dry weight, were present in all stages.

3.3.4.2 Polar lipid classes

The concentration of total polar lipids appeared to increase from stage I to a maximum level at stage II (Table 3.10). The concentration then decreased towards the later stages. Phosphatidylcholine was dominant in the hepatopancreatic polar lipids. Its concentration in earlier maturation stages was higher than in later stages.

3.3.4.3 Fatty acid composition

Palmitic (16:0), palmitoleic (16:1) and oleic (18:1), eicosapentaenoic (20:5ω3) acids always constituted more than 60% of total fatty acids in hepatopancreatic lipids during ovarian maturation (Table 3.11b). The concentrations of saturated and monounsaturated fatty acids decreased 45% and 28% from stage I to IV (Table 3.11a). During the same period, the concentration of total polyunsaturated fatty acids did not change significantly.

Total fatty acids increased by about 14% in spent ovaries.

In both hepatopancreatic neutral and polar lipids, the concentrations of saturated and monounsaturated fatty acids decreased during the ovarian maturation (Tables 3.12a & 3.13a). Polyunsaturated fatty acids did not fluctuate markedly. In addition, the hepatopancreatic lipids contained higher concentrations of eicosapentaenoic (20:5ω3) and docosahexaenoic (22:6ω3) acids than ovarian lipids in all stages studied (Tables 3.6a & 3.11b).

3.3.5 Variation of lipids in muscle

3.3.5.1 Neutral lipid classes

The concentrations of neutral lipids in muscle were much lower than those in the ovary and hepatopancreas, and remained relatively constant during ovarian maturation (Table 3.14). Triglycerides, cholesterol and free fatty acids predominated. Small amounts of diglycerides and monoglycerides, which were less than 1 mg/g tissue dry weight, were present in all stages. Neither one of them varied significantly in concentration as the ovary matured.

3.3.5.2 Polar lipid classes

The concentrations of polar lipids in muscle were lower than those in the hepatopancreas and ovary (Table 3.15). The concentration of total polar lipids, and polar lipid classes remained roughly constant during ovarian maturation.

3.3.5.3 Fatty acid composition

Palmitic (16:0), stearic (18:0), oleic (18:1) and eicosapentaenoic (20:5ω3) acids were the major fatty acids in muscular lipids (Table 3.16b). The concentrations of fatty acids in total muscular lipids were similar among the different stages (Table 3.16a). The concentrations of saturated, monounsaturated and polyunsaturated fatty acid in muscular polar lipids decreased about 40% from stage I to IV while the concentrations of those acids in neutral lipids remained relatively constant (Table 3.17a). Higher proportion of polyunsaturated fatty acids were found in muscular polar lipids than in neutral lipids while the reserve was true for saturated fatty acids (Tables 3.17b & 3.18b).

Table 3.1 Biometric data for female Penaeus chinensis at different ovarian maturation stages.

	Ovarian maturation stage				
	I	II	III	IV	V
Carapace length (cm)	6.1±0.1	5.9±0.2	6.1±0.2	6.0±0.2	6.0±0.2
Total body weight					
wet (g)	117±10	90±10	107±7	105±10	90±5
dry (g)	26±2	23±3	26±1	28±3	23±2
Gonadosomatic index, GSI (%)*	1.9±0.1	2.4±0.2	6.0±0.2	6.3±0.9	2.0±0.4
Hepatosomatic index, HSI (%)	7.6±0.3	8.0±1.1	5.9±0.9	5.9±1.1	6.7±1.1
No. of shrimp	3	3	4	4	5

Values are means ± SE.

* Values are significantly different (1-way ANOVA; $P < 0.05$).

Table 3.2 Total lipid concentrations (% of tissue dry weight) in the ovary, hepatopancreas and muscle during ovarian maturation of Penaeus chinensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Ovary *	12.7±3.4	12.4±2.0	19.1±2.0	38.6±1.6	16.6±1.8
Hepatopancreas	41.3±8.0	43.1±5.8	35.0±1.9	29.4±4.0	28.8±3.1
Muscle	4.9±1.5	5.5±2.1	3.7±0.7	4.2±0.9	4.7±2.2

Values are means ± SE (n = 3 to 5).

* Values are significantly different (1-way ANOVA, $P < 0.05$).

Table 3.3 Absolute amounts of lipid (g) per one shrimp in the ovary, hepatopancreas and muscle during ovarian maturation of Penaeus chinensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Ovary*	0.06±0.02	0.07±0.02	0.29±0.02	1.40±0.20	0.08±0.02
Hepatopancreas*	0.79±0.10	0.77±0.08	0.53±0.07	0.43±0.06	0.47±0.10

Values are means ± SE (n = 3 to 5).

* Values are significantly different (1-way ANOVA, $P < 0.05$).

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Table 3.4 Concentrations of neutral lipid classes (mg lipid per g tissue dry weight) in ovary of Penaeus chinensis during ovarian maturation.

	Ovarian maturation stage				
	I	II	III	IV	V
Monoglycerides	0.83±0.30	1.08±0.62	0.87±0.15	1.28±0.22	0.93±0.23
Diglycerides	0.24±0.03	0.39±0.19	0.62±0.14	0.64±0.15	0.36±0.08
Triglycerides *	36±7	39±12	137±5	139±9	61±14
Cholesterol*	31±9	75±14	88±5	95±5	49±15
Free fatty acids*	44±4	31±9	81±4	92±10	52±14
Total neutral lipids*	112±5	146±26	307±8	298±22	163±24

Values are means ± SE (n = 3 to 5).

The concentration of total neutral lipids is the sum of the concentration of neutral lipid classes.

* Values are significantly different (1-way ANOVA; $P < 0.05$).

Table 3.5 Concentrations of polar lipid classes (mg lipid per g tissue dry weight) in ovary of *Penaeus chinensis* during ovarian maturation.

	Ovarian maturation stage				
	I	II	III	IV	V
Sphingomyelin	17.3±1.3	18.1±1.7	17.4±2.6	17.8±0.7	21.4±5.7
Phosphatidylcholine*	17.8±2.7	54.0±3.1	55.7±3.1	66.3±1.9	31.7±3.2
Phosphatidylserine	13.1±2.0	13.1±1.1	14.5±1.6	19.4±3.2	15.4±5.2
Phosphatidylethanolamine	13.8±1.6	18.9±1.9	21.4±2.4	23.7±2.2	12.6±2.1
Total polar lipids*	71±10	87±4	88±8	144±17	70±12

Values are means ± SE (n = 3 to 5).

The concentration of total polar lipids are determined together with neutral lipids listed in Table 3.4 after separation of lipid classes.

* Values are significantly different (1-way ANOVA; $P < 0.05$).

Table 3.6a Concentrations of ovarian fatty acid content during ovarian maturation of Penaeus chinensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0*	1.26±0.05	2.55±0.18	1.73±0.04	1.78±0.12	0.64±0.08
16:0*	14.1±0.6	16.7±0.6	24.2±0.5	22.6±1.1	14.3±0.9
18:0	2.89±0.23	2.54±0.54	2.98±0.13	2.30±0.23	2.82±0.34
20:0*	0.83±0.12	0.47±0.10	0.94±0.13	0.64±0.02	0.34±0.09
Total*	19.1±0.7	22.3±0.8	29.9±0.5	29.3±1.3	18.1±1.1
Monounsaturated					
16:1*	8.91±0.38	12.1±0.7	15.0±0.6	19.6±1.6	7.34±0.21
18:1*	18.6±1.1	18.3±0.9	28.6±1.1	24.9±1.3	15.4±0.8
20:1	0.31±0.06	0.36±0.03	0.28±0.05	0.30±0.09	0.34±0.06
Total*	27.8±1.2	30.8±1.4	43.9±1.4	44.8±2.9	23.1±0.9
PUFA (ω6)**					
18:2*	0.29±0.08	0.81±0.03	1.09±0.23	0.81±0.07	0.84±0.05
20:2*	0.86±0.08	0.52±0.06	0.81±0.03	1.17±0.10	0.75±0.04
20:4*	1.67±0.11	1.69±0.02	2.37±0.08	3.09±0.05	1.07±0.06
PUFA (ω3)***					
18:3*	0.85±0.03	0.91±0.07	0.48±0.03	0.40±0.03	0.15±0.06
18:4*	0.11±0.02	0.15±0.04	0.49±0.08	0.56±0.11	0.35±0.04
20:5*	4.18±0.26	4.28±0.36	6.25±0.12	9.98±0.68	3.82±0.27
22:5*	0.60±0.04	0.61±0.05	0.45±0.09	0.92±0.08	0.35±0.09
22:6*	1.93±0.23	1.19±0.28	2.34±0.42	5.31±0.26	2.43±0.30
Total PUFA*	10.5±0.4	10.2±0.7	14.3±0.5	22.2±1.0	9.8±0.5
Total*	57.4±0.7	63.2±2.3	88.0±1.5	94.4±4.4	50.9±1.9

Data are means ± SE (n = 3 to 5), and expressed as mg fatty acid/g tissue dry weight.

* Values are significantly different (1-way ANOVA; $P < 0.05$).

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

Table 3.6b Relative amounts of total ovarian fatty acid content during ovarian maturation of Penaeus chinensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0	2.2%	4.0%	2.0%	1.9%	1.3%
16:0	24.6%	26.4%	27.5%	24.0%	28.1%
18:0	5.0%	4.0%	3.4%	2.4%	5.5%
20:0	1.4%	0.7%	1.1%	0.7%	0.7%
Total	33%	35%	34%	29%	36%
Monounsaturated					
16:1	15.5%	19.2%	17.0%	20.8%	14.4%
18:1	32.4%	29.0%	32.5%	26.4%	30.2%
20:1	0.5%	0.6%	0.3%	0.3%	0.7%
Total	49%	49%	50%	48%	45%
PUFA (ω6)**					
18:2	0.5%	1.3%	1.2%	0.9%	1.6%
20:2	1.5%	0.8%	0.9%	1.2%	1.5%
20:4	2.9%	2.7%	2.7%	3.3%	2.1%
PUFA (ω3)***					
18:3	1.5%	1.4%	0.5%	0.4%	0.3%
18:4	0.2%	0.2%	0.6%	0.6%	0.7%
20:5	7.3%	6.8%	7.1%	10.6%	7.5%
22:5	1.0%	1.0%	0.5%	1.0%	0.7%
22:6	3.4%	1.9%	2.7%	5.6%	4.8%
Total PUFA	18%	16%	16%	23%	19%

Data are means and expressed as % of total fatty acids.

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

Table 3.7a Concentrations of fatty acid content in ovarian neutral lipids during ovarian maturation of Penaeus chinensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0*	0.56±0.05	0.63±0.05	1.28±0.04	1.23±0.09	0.55±0.09
16:0*	6.63±0.25	9.69±0.45	17.9±0.2	14.7±0.3	10.2±0.7
18:0*	1.36±0.16	1.47±0.06	2.21±0.22	15.0±0.1	2.00±0.11
20:0*	0.39±0.09	0.37±0.07	0.70±0.07	0.62±0.05	0.34±0.03
Total*	8.9±0.2	12.2±0.4	22.1±0.3	18.0±0.3	13.1±0.9
Monounsaturated					
16:1*	5.19±0.10	8.02±0.27	12.1±0.3	13.7±0.5	6.21±0.26
18:1*	7.74±0.17	9.61±0.18	20.2±0.4	15.2±0.8	9.93±0.61
20:1	0.15±0.02	0.21±0.03	0.20±0.02	0.20±0.02	0.24±0.03
Total*	13.1±0.3	17.8±0.3	32.5±0.5	29.1±0.8	16.4±0.7
PUFA (ω6)**					
18:2*	0.14±0.02	0.47±0.06	0.81±0.05	0.53±0.07	0.63±0.07
20:2*	0.22±0.03	0.34±0.05	0.51±0.07	0.76±0.05	0.53±0.12
20:4*	0.78±0.05	0.98±0.07	1.75±0.17	2.01±0.14	0.96±0.08
PUFA (ω3)***					
18:3*	0.24±0.03	0.53±0.06	0.36±0.04	0.26±0.03	0.22±0.05
18:4*	0.15±0.03	0.23±0.05	0.36±0.03	0.33±0.03	0.25±0.05
20:5*	1.86±0.24	2.38±0.35	4.53±0.28	6.09±0.20	2.71±0.36
22:5*	0.38±0.01	0.35±0.07	0.35±0.09	0.60±0.03	0.35±0.06
22:6*	0.81±0.03	0.69±0.13	1.64±0.16	2.45±0.11	1.37±0.14
Total PUFA*	4.6±0.2	6.0±0.3	10.3±0.4	13.0±0.3	7.0±0.7
Total*	26.6±0.4	36.0±0.4	64.9±0.6	60.2±1.0	36.5±1.7

Data are means ± SE (n = 3 to 5), and expressed as mg fatty acid/g tissue dry weight.

* Values are significantly different (1-way ANOVA; $P < 0.05$).

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

Table 3.7b Relative amounts of fatty acid content in ovarian neutral lipids during ovarian maturation of Penaeus chinensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0	2.1%	1.8%	2.0%	2.0%	1.5%
16:0	24.9%	26.9%	27.6%	24.4%	27.9%
18:0	5.1%	4.1%	3.4%	2.5%	5.5%
20:0	1.5%	1.0%	1.1%	1.0%	0.9%
Total	34%	34%	34%	30%	36%
Monounsaturated					
16:1	19.5%	22.3%	18.7%	22.8%	17.0%
18:1	29.1%	26.7%	31.1%	25.2%	27.2%
20:1	0.5%	0.6%	0.3%	0.3%	0.7%
Total	49%	50%	50%	48%	45%
PUFA (ω6)**					
18:2	0.5%	1.3%	1.2%	0.9%	1.7%
20:2	0.8%	0.9%	0.8%	1.3%	1.5%
20:4	3.0%	2.7%	2.7%	3.3%	2.6%
PUFA (ω3)**					
18:3	0.9%	1.5%	0.5%	0.4%	0.6%
18:4	0.6%	0.6%	0.6%	0.5%	0.7%
20:5	7.0%	6.6%	7.0%	10.1%	7.4%
22:5	1.4%	1.0%	0.5%	1.0%	1.0%
22:6	3.0%	1.9%	2.5%	4.1%	3.8%
Total PUFA	17%	16%	16%	22%	19%

Data are means and expressed as % of total fatty acids.

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

Table 3.8a Concentrations of fatty acid content in ovarian polar lipids during ovarian maturation of Penaeus chinensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0*	0.53±0.01	0.47±0.02	0.56±0.05	0.67±0.03	0.35±0.04
16:0*	6.91±0.14	6.35±0.50	5.32±0.34	7.01±0.11	3.43±0.21
18:0	1.42±0.17	1.97±0.29	1.66±0.24	1.71±0.14	1.68±0.09
20:0*	0.41±0.07	0.18±0.05	0.21±0.02	0.20±0.02	0.08±0.02
Total*	9.3±0.3	9.0±0.7	7.8±0.4	9.6±0.6	5.6±0.3
Monounsaturated					
16:1*	4.37±0.16	4.60±0.44	3.30±0.19	6.08±0.10	1.76±0.16
18:1*	9.11±0.25	6.95±0.23	6.29±0.26	7.72±0.11	3.69±0.13
20:1*	0.15±0.01	0.14±0.02	0.06±0.01	0.09±0.01	0.08±0.02
Total*	13.6±0.3	11.7±0.5	9.7±0.1	13.9±0.2	5.5±0.3
PUFA (ω6)**					
18:2*	0.24±0.04	0.31±0.03	0.24±0.01	0.25±0.03	0.20±0.02
20:2	0.42±0.03	0.20±0.02	0.18±0.03	0.36±0.03	0.18±0.02
20:4*	0.76±0.04	0.66±0.03	0.72±0.03	0.96±0.13	0.53±0.03
PUFA (ω3)***					
18:3*	0.04±0.02	0.12±0.03	0.11±0.01	0.34±0.05	0.14±0.02
18:4*	0.05±0.03	0.06±0.03	0.11±0.02	0.17±0.03	0.08±0.01
20:5*	2.15±0.27	1.73±0.23	1.68±0.16	3.19±0.16	0.82±0.05
22:5*	0.13±0.03	0.11±0.03	0.24±0.03	0.38±0.03	0.07±0.01
22:6*	0.44±0.04	0.45±0.08	0.51±0.03	1.65±0.24	0.58±0.05
Total PUFA	4.2±0.3	3.6±0.2	3.8±0.2	7.3±0.4	2.6±0.5
Total*	27.1±0.9	24.3±0.5	21.2±0.5	30.8±0.7	13.7±0.8

Data are means ± SE (n = 3 to 5), and expressed as mg fatty acid/g tissue dry weight.

* Values are significantly different (1-way ANOVA; $P < 0.05$).

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

Table 3.8b Relative amounts of fatty acid content in ovarian polar lipids during ovarian maturation of Penaeus chinensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0	2.0%	1.9%	2.6%	2.2%	2.6%
16:0	25.5%	26.1%	25.1%	22.8%	25.1%
18:0	5.2%	8.1%	7.8%	5.6%	12.3%
20:0	1.5%	0.7%	1.0%	0.6%	0.6%
Total	34%	37%	37%	31%	41%
Monounsaturated					
16:1	16.1%	18.9%	15.6%	19.7%	12.9%
18:1	33.6%	28.6%	29.7%	25.1%	27.0%
20:1	0.6%	0.6%	0.3%	0.3%	0.6%
Total	50%	48%	45%	45%	40%
PUFA (ω6)**					
18:2	0.9%	1.3%	1.1%	0.8%	1.5%
20:2	1.6%	0.8%	0.8%	1.2%	1.3%
20:4	2.8%	2.7%	3.4%	3.1%	3.9%
PUFA (ω3)***					
18:3	0.1%	0.5%	0.5%	1.1%	1.0%
18:4	0.2%	0.2%	0.5%	0.6%	0.6%
20:5	7.9%	7.1%	7.9%	10.4%	6.0%
22:5	0.5%	0.5%	1.1%	1.2%	0.5%
22:6	1.6%	1.9%	2.4%	5.3%	4.3%
Total PUFA	16%	15%	18%	24%	19%

Data are means and expressed as % of total fatty acids.

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

ble 3.9 Concentrations of neutral lipid classes (mg lipid per g tissue dry weight) in hepatopancreas of Penaeus chinensis during ovarian maturation.

	Ovarian maturation stage				
	I	II	III	IV	V
Monoglycerides	1.18±0.47	1.49±0.36	1.16±0.35	0.90±0.19	1.03±0.33
Diglycerides	0.48±0.18	0.59±0.11	0.44±0.12	0.76±0.17	1.06±0.42
Triglycerides*	181±30	211±24	135±21	102±4	141±9
Cholesterol*	12±2	27±4	54±5	43±1	45±3
Free fatty acids*	92±29	123±32	40±9	34±4	53±14
Total neutral lipids*	286±16	363±22	230±30	180±9	240±18

Values are means ± SE (n = 3 to 5).

The concentration of total neutral lipids is the sum of the concentrations of neutral lipid classes.

* Values are significantly different (1-way ANOVA; $P < 0.05$).

Table 3.10 Concentrations of polar lipid classes (mg lipid per g tissue dry weight) in hepatopancreas of Penaeus chinensis during ovarian maturation.

	Ovarian maturation stage				
	I	II	III	IV	V
Sphingomyelin	16.4±1.7	18.1±4.1	17.4±2.3	15.4±1.7	16.1±0.7
Phosphatidylcholine*	60.6±1.6	66.8±0.8	51.7±2.7	46.7±3.8	53.5±6.7
Phosphatidylserine	21.7±1.4	20.5±3.8	16.1±1.4	18.1±4.8	16.8±6.0
Phosphatidylethanolamine	9.1±0.8	23.3±1.9	25.1±3.5	18.1±6.7	18.5±5.8
Total polar lipids	79±19	127±35	93±26	86±4	71±13

Values are means ± SE (n = 3 to 5).

The concentration of total polar lipids are determined together with neutral lipids listed in Table 3.4 after separation of lipid classes.

* Values are significantly different (1-way ANOVA; $P < 0.05$).

Table 3.11a Concentrations of hepatopancreatic fatty acid content during ovarian maturation of Penaeus chinensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0*	7.07±0.11	9.56±0.25	8.24±0.18	6.49±0.40	8.94±0.15
16:0*	47.0±0.2	39.7±0.9	37.4±0.2	23.5±0.4	48.8±0.1
18:0*	15.4±0.3	13.3±0.4	14.5±0.4	8.08±0.12	12.5±0.3
20:0*	0.69±0.03	0.76±0.04	0.53±0.06	0.53±0.03	0.87±0.02
Total*	70.1±0.6	63.3±1.5	60.6±0.8	38.6±0.8	71.1±0.4
Monounsaturated					
16:1*	27.0±0.4	22.9±0.3	21.7±0.4	16.7±0.1	21.6±0.1
18:1*	19.3±0.3	17.8±0.3	18.0±0.4	17.1±0.1	16.7±0.2
20:1*	0.43±0.06	0.17±0.04	0.23±0.06	0.67±0.03	0.28±0.03
Total*	46.7±0.4	40.9±0.6	39.9±0.8	34.4±0.1	38.6±0.3
PUFA (ω6)**					
18:2	2.58±0.15	2.86±0.15	1.98±0.21	2.37±0.19	2.49±0.17
20:2	3.85±0.13	3.16±0.20	3.67±0.19	3.25±0.17	3.67±0.14
20:4	5.89±0.27	6.19±0.13	6.31±0.17	6.28±0.22	5.99±0.14
PUFA (ω3)***					
18:3*	5.01±0.11	4.27±0.06	4.47±0.13	5.68±0.19	7.99±0.20
18:4*	0.61±0.06	0.59±0.05	0.67±0.04	0.32±0.06	0.63±0.03
20:5*	19.5±0.3	17.1±0.2	17.3±0.1	18.0±0.3	15.8±0.2
22:5*	2.84±0.13	3.45±0.16	3.91±0.15	3.87±0.11	3.98±0.18
22:6*	6.10±0.19	6.57±0.20	8.38±0.23	8.77±0.18	8.38±0.12
Total PUFA	46.4±1.0	44.1±1.1	46.6±1.0	48.6±1.2	49.0±1.1
Total*	163±2	148±3	147±2	122±2	159±2

Data are means ± SE (n = 3 to 5), and expressed as mg fatty acid/g tissue dry weight.

* Values are significantly different (1-way ANOVA; $P < 0.05$).

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

Table 3.11b Relative amounts of total hepatopancreatic fatty acid content during ovarian maturation of Penaeus chinensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0	4.3%	6.4%	5.6%	5.3%	5.6%
16:0	28.8%	26.7%	25.4%	19.3%	30.7%
18:0	9.4%	9.0%	9.9%	6.6%	7.9%
20:0	0.4%	0.5%	0.4%	0.4%	0.5%
Total	43%	42%	41%	32%	45%
Monounsaturated					
16:1	16.5%	15.4%	14.8%	13.7%	13.6%
18:1	11.8%	12.0%	12.2%	14.0%	10.5%
20:1	0.3%	0.1%	0.2%	0.6%	0.2%
Total	29%	28%	27%	28%	24%
PUFA (ω6)**					
18:2	1.6%	1.9%	1.3%	1.9%	1.6%
20:2	2.4%	2.1%	2.5%	2.7%	2.3%
20:4	3.6%	4.2%	4.3%	5.2%	3.8%
PUFA (ω3)***					
18:3	3.1%	2.9%	3.0%	4.7%	5.0%
18:4	0.4%	0.4%	0.5%	0.3%	0.4%
20:5	11.9%	11.5%	11.7%	14.8%	10.0%
22:5	1.7%	2.3%	2.7%	3.2%	2.5%
22:6	3.7%	4.4%	5.7%	7.2%	5.3%
Total PUFA	28%	30%	32%	40%	31%

Data are means and expressed as % of total fatty acids.

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

Table 3.12a Concentrations of fatty acid content in hepatopancreatic neutral lipids during ovarian maturation of Penaeus chinensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0*	4.95±0.15	7.06±0.15	6.18±0.08	4.41±0.06	6.26±0.07
16:0*	28.9±0.2	27.7±0.2	26.0±0.2	12.0±0.1	30.1±0.1
18:0*	10.8±0.2	10.7±0.1	10.9±0.1	5.49±0.14	8.77±0.14
20:0*	0.48±0.03	0.51±0.02	0.40±0.02	0.36±0.04	0.61±0.03
Total*	45.1±0.3	46.0±0.2	42.5±0.3	22.3±0.2	45.8±0.2
Monounsaturated					
16:1*	18.9±0.2	18.3±0.2	13.3±0.2	11.4±0.2	15.1±0.2
18:1*	13.5±0.2	14.3±0.2	13.5±0.2	11.6±0.1	11.7±0.2
20:1*	0.30±0.04	0.28±0.02	0.17±0.01	0.46±0.03	0.20±0.02
Total*	32.7±0.2	32.9±0.1	27.0±0.3	23.4±0.2	27.0±0.3
PUFA (ω6)**					
18:2*	1.81±0.18	2.29±0.19	1.69±0.01	1.61±0.03	1.74±0.15
20:2*	2.70±0.12	2.53±0.03	2.75±0.11	2.21±0.06	2.57±0.10
20:4*	4.12±0.11	4.95±0.10	4.95±0.12	4.67±0.13	4.19±0.12
PUFA (ω3)***					
18:3*	3.51±0.04	3.22±0.03	3.35±0.13	3.86±0.04	5.59±0.18
18:4*	0.43±0.03	0.47±0.02	0.50±0.04	0.32±0.01	0.44±0.03
20:5*	12.6±0.1	11.8±0.1	11.9±0.1	10.5±0.1	11.8±0.1
22:5*	1.99±0.03	2.76±0.12	2.93±0.07	2.33±0.15	2.79±0.09
22:6*	4.27±0.16	5.26±0.03	6.29±0.12	6.06±0.07	5.87±0.15
Total PUFA*	31.5±0.3	33.3±0.3	34.4±0.2	31.6±0.1	35.0±0.5
Total*	109±0.4	112±0.3	103±1.2	77±0.4	108±1

Data are means ± SE (n = 3 to 5), and expressed as mg fatty acid/g tissue dry weight.

* Values are significantly different (1-way ANOVA; $P < 0.05$).

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

Table 3.12b Relative amounts of fatty acid content in hepatopancreatic neutral lipids during ovarian maturation of Penaeus chinensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0	4.5%	6.3%	6.0%	5.7%	5.8%
16:0	26.4%	24.7%	24.1%	15.5%	28.0%
18:0	9.9%	9.5%	10.5%	7.1%	8.1%
20:0	0.4%	0.5%	0.4%	0.5%	0.6%
Total	41%	41%	41%	29%	43%
Monounsaturated					
16:1	17.3%	16.3%	12.8%	14.7%	14.1%
18:1	12.4%	12.7%	13.0%	15.0%	10.8%
20:1	0.3%	0.2%	0.2%	0.6%	0.2%
Total	30%	29%	26%	30%	25%
PUFA (ω6)**					
18:2	1.7%	2.0%	1.6%	5.0%	1.6%
20:2	2.5%	2.3%	2.7%	2.9%	2.4%
20:4	3.8%	4.4%	4.8%	6.0%	3.9%
PUFA (ω3)***					
18:3	3.2%	2.9%	3.2%	5.0%	5.2%
18:4	0.4%	0.4%	0.5%	0.4%	0.4%
20:5	11.6%	10.5%	11.5%	13.6%	10.9%
22:5	1.8%	2.5%	2.8%	3.0%	2.6%
22:6	3.9%	4.7%	6.1%	7.8%	5.4%
Total PUFA	29%	30%	33%	41%	32%

Data are means and expressed as % of total fatty acids.

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

Table 3.13a Concentrations of fatty acid content in hepatopancreatic polar lipids during ovarian maturation of Penaeus chinensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0*	1.91±0.08	1.72±0.07	1.65±0.06	1.95±0.08	2.68±0.11
16:0*	12.7±0.2	15.1±0.2	12.5±0.2	7.06±0.11	10.6±0.2
18:0*	7.15±0.09	10.2±0.2	6.90±0.15	4.41±0.10	3.76±0.16
20:0*	0.19±0.03	0.14±0.03	0.11±0.01	0.16±0.02	0.26±0.03
Total*	21.9±0.3	27.2±0.4	21.1±0.4	13.6±0.3	17.3±0.5
Monounsaturated					
16:1*	9.28±0.14	9.12±0.08	4.35±0.18	4.01±0.08	6.49±0.13
18:1*	7.22±0.07	8.21±0.17	3.59±0.16	3.12±0.06	5.00±0.22
20:1*	0.12±0.02	0.03±0.01	0.05±0.03	0.20±0.04	0.08±0.03
Total*	16.6±0.2	17.4±0.3	8.0±0.4	7.3±0.2	11.6±0.3
PUFA (ω6)**					
18:2*	0.70±0.07	0.51±0.04	0.40±0.07	0.71±0.02	0.75±0.07
20:2*	1.04±0.02	0.57±0.04	0.73±0.01	0.98±0.05	1.10±0.20
20:4	2.59±0.03	2.11±0.07	2.26±0.07	2.88±0.15	2.80±0.18
PUFA (ω3)***					
18:3*	1.35±0.17	0.77±0.05	0.89±0.06	1.70±0.07	2.40±0.12
18:4*	0.16±0.02	0.11±0.03	0.13±0.02	0.10±0.05	0.19±0.04
20:5*	5.26±0.14	3.07±0.11	3.45±0.14	5.41±0.11	4.75±0.08
22:5*	0.77±0.04	0.62±0.05	0.78±0.06	1.16±0.12	1.19±0.09
22:6*	1.65±0.04	1.18±0.07	1.68±0.06	2.63±0.09	2.51±0.11
Total PUFA*	13.5±0.5	8.9±0.5	10.3±0.3	15.6±0.5	15.7±0.8
Total*	52.1±1.0	53.5±1.1	39.4±1.1	36.5±1.0	44.6±1.8

Data are means ± SE (n = 3 to 5), and expressed as mg fatty acid/g tissue dry weight.

* Values are significantly different (1-way ANOVA; $P < 0.05$).

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

Table 3.13b Relative amounts of fatty acid content in hepatopancreatic polar lipids during ovarian maturation of Penaeus chinensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0	3.7%	3.2%	4.2%	5.3%	6.0%
16:0	24.4%	28.3%	31.6%	19.4%	23.8%
18:0	13.7%	19.1%	17.5%	12.1%	8.4%
20:0	0.4%	0.3%	0.3%	0.4%	0.6%
Total	42%	51%	54%	37%	39%
Monounsaturated					
16:1	17.8%	17.0%	11.0%	11.0%	14.6%
18:1	13.9%	15.3%	9.1%	8.6%	12.2%
20:1	0.2%	0.1%	0.1%.	0.6%	0.2%
Total	32%	32%	20%	20%	26%
PUFA (ω6)**					
18:2	1.3%	1.0%	1.0%	1.9%	1.7%
20:2	2.0%	1.1%	1.9%	2.7%	2.5%
20:4	5.0%	3.9%	5.7%	7.9%	6.3%
PUFA (ω3)***					
18:3	2.6%	1.4%	2.3%	4.7%	5.4%
18:4	0.3%	0.2%	0.3%	0.3%	0.4%
20:5	10.1%	5.7%	8.7%	14.8%	10.6%
22:5	1.5%	1.2%	2.0%	3.2%	2.7%
22:6	3.2%	2.2%	4.2%	7.2%	5.6%
Total PUFA	26%	17%	26%	43%	35%

Data are means and expressed as % of total fatty acids.

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

Table 3.14 Concentrations of neutral lipid classes (mg lipid per g tissue dry weight) in muscle of Penaeus chinensis during ovarian maturation.

	Ovarian maturation stage				
	I	II	III	IV	V
Monglycerides	0.30±0.12	0.39±0.16	0.51±0.05	0.34±0.11	0.32±0.14
Diglycerides	0.14±0.09	0.33±0.17	0.15±0.08	0.20±0.10	0.21±0.41
Triglycerides	15±3	17±7	40±12	29±10	15±4
Cholesterol	22±9	31±13	32±7	24±11	40±15
Free fatty acids	24±10	24±0.3	22±7	22±9	19±8
Total neutral lipids	61±9	73±7	95±6	75±8	75±9

Values are means ± SE (n = 3 to 5).

The concentration of total neutral lipids is the sum of the concentrations of neutral lipid classes.

* Values are significantly different (1-way ANOVA; $P < 0.05$).

Table 3.15 Concentrations of polar lipid classes (mg lipid per g tissue dry weight) in muscle of Penaeus chinensis during ovarian maturation.

	Ovarian maturation stage				
	I	II	III	IV	V
Sphingomyelin	3.5±0.5	2.2±0.9	3.2±0.8	3.2±0.9	2.7±0.2
Phosphatidylcholine	13±3	11±3	13±1	11±1	13±2
Phosphatidylserine	4.2±1.3	3.5±2.4	3.8±1.1	3.5±1.5	4.1±1.2
Phosphatidylethanolamine	6.3±2.3	6.9±1.7	6.7±0.2	5.0±1.5	4.7±1.8
Total polar lipids	29±7	36±6	26±8	16±1	22±10

Values are means ± SE (n = 3 to 5).

The concentration of total polar lipids are determined together with neutral lipids listed in Table 3.4 after separation of lipid classes.

• Values are significantly different (1-way ANOVA; $P < 0.05$).

Table 3.16a Concentrations of muscular fatty acid content during ovarian maturation of Penaeus chinensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0*	0.88±0.10	0.93±0.03	0.99±0.02	1.06±0.04	0.84±0.04
16:0*	6.75±0.08	6.55±0.20	6.55±0.12	7.85±0.07	7.50±0.05
18:0*	6.90±0.10	7.55±0.15	6.95±0.08	6.95±0.14	6.56±0.10
20:0*	2.65±0.07	2.36±0.09	2.72±0.05	1.68±0.09	2.11±0.09
Total	17.2±0.4	17.4±0.5	17.2±0.2	17.6±0.3	17.1±0.3
Monounsaturated					
16:1*	3.76±0.05	3.83±0.09	4.19±0.07	3.64±0.13	4.06±0.06
18:1*	7.25±0.10	6.95±0.05	7.50±0.10	7.56±0.86	7.05±0.09
20:1*	0.11±0.02	0.18±0.03	0.18±0.02	0.22±0.04	0.11±0.01
Total*	11.1±0.2	11.0±0.2	11.9±0.2	11.5±0.3	11.2±0.2
PUFA (ω6)**					
18:2	0.47±0.02	0.48±0.08	0.41±0.03	0.36±0.03	0.53±0.06
20:2	0.19±0.02	0.14±0.01	0.19±0.02	0.13±0.02	0.18±0.02
20:4	2.77±0.11	2.68±0.05	2.52±0.06	2.61±0.11	2.79±0.06
PUFA (ω3)***					
18:3*	0.17±0.01	0.24±0.01	0.21±0.01	0.29±0.01	0.23±0.02
18:4*	0.32±0.01	0.41±0.02	0.40±0.02	0.42±0.01	0.41±0.02
20:5*	5.20±0.08	4.68±0.13	5.45±0.05	5.50±0.05	5.35±0.08
22:5	0.29±0.04	0.30±0.03	0.28±0.01	0.27±0.02	0.29±0.03
22:6*	4.31±0.24	4.05±0.05	4.07±0.06	4.56±0.10	4.20±0.09
Total PUFA	13.7±0.5	13.0±0.4	13.5±0.3	14.1±0.4	14.0±0.4
Total	42.0±1.1	41.3±1.1	4.26±0.4	43.1±0.7	42.3±0.8

Data are means ± SE (n = 3 to 5), and expressed as mg fatty acid/g tissue dry weight.

* Values are significantly different (1-way ANOVA; $P < 0.05$).

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

Table 3.16b Relative amounts of total muscular fatty acid content during ovarian maturation of Penaeus chinensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0	2.1%	2.2%	2.3%	2.5%	2.0%
16:0	16.1%	15.8%	15.5%	18.2%	17.8%
18:0	16.4%	18.3%	16.3%	16.1%	15.6%
20:0	6.3%	5.7%	6.4%	3.9%	5.0%
Total	41%	42%	40%	41%	40%
Monounsaturated					
16:1	8.9%	9.3%	9.8%	8.4%	9.6%
18:1	17.2%	16.8%	17.6%	17.6%	16.7%
20:1	0.3%	0.4%	0.4%	0.5%	0.3%
Total	26%	27%	28%	26%	28%
PUFA (ω6)**					
18:2	1.1%	1.2%	1.0%	0.8%	1.2%
20:2	0.4%	0.3%	0.4%	0.3%	0.4%
20:4	6.6%	6.5%	5.9%	6.0%	6.6%
PUFA (ω3)***					
18:3	0.4%	0.6%	0.5%	0.7%	0.5%
18:4	0.8%	1.0%	0.9%	1.0%	1.0%
20:5	12.4%	11.3%	12.8%	12.7%	12.7%
22:5	0.7%	0.7%	0.6%	0.6%	0.7%
22:6	10%	9.8%	9.6%	10.6%	9.9%
Total PUFA	33%	31%	32%	33%	33%

Data are means and expressed as % of total fatty acids.

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

Table 3.17a Concentrations of fatty acid content in muscular neutral lipids during ovarian maturation of Penaeus chinensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0*	1.07±0.07	1.09±0.07	1.26±0.02	1.37±0.10	1.12±0.07
16:0*	4.39±0.10	4.84±0.08	5.04±0.18	6.45±0.12	5.55±0.07
18:0*	4.49±0.08	4.84±0.06	5.35±0.11	5.70±0.08	5.39±0.13
20:0*	1.73±0.05	1.51±0.03	2.09±0.07	1.88±0.06	1.56±0.06
Total*	1.73±0.3	11.6±0.3	13.8±0.3	15.2±0.6	13.6±0.3
Monounsaturated					
16:1*	2.44±0.06	2.45±0.07	3.23±0.05	2.98±0.09	3.00±0.05
18:1*	4.71±0.05	4.44±0.08	5.75±0.06	6.25±0.05	5.38±0.05
20:1*	0.12±0.02	0.12±0.02	0.14±0.02	0.18±0.01	0.13±0.02
Total*	7.3±0.1	7.0±0.2	9.2±0.2	9.4±0.2	8.4±0.2
PUFA (ω6)**					
18:2*	0.33±0.03	0.31±0.03	0.31±0.03	0.34±0.01	0.39±0.01
20:2*	0.12±0.02	0.09±0.02	0.14±0.02	0.11±0.02	0.14±0.01
20:4	1.80±0.07	1.71±0.09	1.94±0.04	2.14±0.09	2.07±0.07
PUFA (ω3)***					
18:3	0.16±0.01	0.18±0.06	0.16±0.01	0.19±0.01	0.17±0.01
18:4*	0.21±0.02	0.16±0.06	0.26±0.01	0.16±0.01	0.21±0.02
20:5	2.89±0.12	2.61±0.18	2.68±0.08	2.99±0.09	2.82±0.10
22:5*	0.16±0.01	0.14±0.01	0.16±0.01	0.17±0.01	0.11±0.01
22:6*	2.30±0.06	2.09±0.07	2.63±0.09	2.74±0.05	2.61±0.11
Total PUFA	8.0±0.4	7.3±0.1	8.3±0.3	8.9±0.3	8.5±0.3
Total*	26.7±0.7	25.9±0.5	31.2±0.7	33.6±1.0	30.5±0.7

Data are means ± SE (n = 3 to 5), and expressed as mg fatty acid/g tissue dry weight.

* Values are significantly different (1-way ANOVA; $P < 0.05$).

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

Table 3.17b Relative amounts of fatty acid content in muscular neutral lipids during ovarian maturation of Penaeus chinensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0	4.0%	4.2%	4.0%	4.1%	3.7%
16:0	16.3%	16.1%	16.3%	19.2%	18.2%
18:0	16.7%	18.7%	17.1%	16.9%	18.2%
20:0	6.4%	5.8%	6.7%	5.6%	5.1%
Total	43%	45%	44%	46%	45%
Monounsaturated					
16:1	9.1%	9.5%	10.4%	8.9%	9.9%
18:1	17.5%	17.1%	18.5%	18.5%	17.1%
20:1	0.4%	0.4%	0.4%	0.5%	0.4%
Total	27%	27%	29%	28%	27%
PUFA (ω6)**					
18:2	0.6%	0.7%	0.5%	0.6%	0.5%
20:2	0.4%	0.3%	0.5%	0.3%	0.4%
20:4	6.7%	6.6%	6.2%	6.4%	6.8%
PUFA (ω3)***					
18:3	0.6%	0.7%	0.5%	0.6%	0.5%
18:4	0.8%	0.6%	0.8%	0.5%	0.6%
20:5	10.7%	10.1%	8.6%	8.9%	9.2%
22:5	0.6%	0.5%	0.5%	0.5%	0.4%
22:6	8.6%	8.1%	8.4%	8.2%	8.6%
Total PUFA	30%	28%	27%	26%	27%

Data are means and expressed as % of total fatty acids.

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

Table 3.18a Concentrations of fatty acid content in muscular polar lipids during ovarian maturation of Penaeus chinensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0	0.22±0.02	0.23±0.01	0.21±0.02	0.22±0.02	0.15±0.03
16:0*	2.03±0.06	1.96±0.07	1.38±0.04	1.26±0.05	1.50±0.08
18:0*	2.07±0.07	2.27±0.06	1.46±0.08	1.11±0.05	1.32±0.03
20:0*	0.80±0.05	0.71±0.04	0.57±0.02	0.27±0.02	0.42±0.01
Total*	5.1±0.2	5.3±0.2	3.6±0.2	2.9±0.1	3.4±0.2
Monounsaturated					
16:1*	1.13±0.07	1.15±0.07	0.88±0.05	0.58±0.06	0.81±0.07
18:1*	2.17±0.07	2.08±0.08	1.58±0.06	1.22±0.04	1.41±0.09
20:1	0.01±0.01	0.02±0.01	0.02±0.01	0.02±0.01	0.03±0.01
Total*	3.3±0.2	3.3±0.2	2.5±0.1	1.8±0.1	2.3±0.1
PUFA (ω6)**					
18:2	0.09±0.02	0.10±0.01	0.09±0.01	0.11±0.01	0.12±0.02
20:2	0.11±0.01	0.14±0.01	0.14±0.01	0.12±0.01	0.14±0.01
20:4*	0.83±0.06	0.80±0.10	0.53±0.03	0.42±0.05	0.56±0.04
PUFA (ω3)***					
18:3*	0.06±0.01	0.07±0.02	0.07±0.01	0.10±0.01	0.05±0.01
18:4	0.12±0.02	0.12±0.01	0.14±0.01	0.12±0.02	0.13±0.01
20:5*	2.06±0.07	1.91±0.09	1.64±0.05	1.88±0.05	1.57±0.04
22:5*	0.10±0.01	0.11±0.01	0.11±0.01	0.14±0.02	0.08±0.01
22:6*	1.29±0.08	1.22±0.01	0.86±0.04	0.73±0.04	0.84±0.06
Total PUFA*	4.7±0.5	4.5±0.3	3.6±0.2	3.6±0.2	3.5±0.2
Total*	13.1±0.7	12.9±0.6	9.7±0.4	8.3±0.4	9.1±0.5

Data are means ± SE (n = 3 to 5), and expressed as mg fatty acid/g tissue dry weight.

* Values are significantly different (1-way ANOVA; $P < 0.05$).

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

Table 3.18b Relative amounts of fatty acid content in muscular polar lipids during ovarian maturation of Penaeus chinensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0	1.6%	1.8%	2.2%	2.7%	1.6%
16:0	15.5%	15.2%	14.3%	15.2%	16.5%
18:0	15.9%	17.6%	15.1%	13.4%	14.5%
20:0	6.1%	5.5%	5.9%	3.2%	4.6%
Total	39%	40%	38%	35%	37%
Monounsaturated					
16:1	8.6%	8.9%	9.1%	7.0%	8.9%
18:1	16.6%	17.6%	16.3%	14.7%	15.5%
20:1	0.1%	0.1%	0.2%	0.2%	0.3%
Total	25%	25%	25%	22%	25%
PUFA (ω6)**					
18:2	0.7%	0.7%	0.9%	1.3%	1.3%
20:2	0.8%	1.1%	1.5%	1.5%	1.5%
20:4	6.4%	6.2%	5.5%	5.0%	6.1%
PUFA (ω3)***					
18:3	0.4%	0.5%	0.7%	1.1%	0.5%
18:4	0.9%	0.9%	1.4%	1.4%	1.4%
20:5	15.8%	14.8%	17.0%	22.7%	17.2%
22:5	0.8%	0.8%	1.1%	1.7%	0.9%
22:6	9.9%	9.4%	8.9%	8.8%	9.2%
Total PUFA	36%	35%	37%	43%	38%

Data are means and expressed as % of total fatty acids.

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

3.4 Discussion

3.4.1 Variation of lipids in ovary during ovarian maturation

As the ovary matured, the gonadosomatic index of Penaeus chinensis increased three folds from immature stage to mature stage, and decreases in spent ovaries. Similar results have been reported in other penaeids, such as P. japonicus (Teshima & Kanazawa, 1983), P. kerathurus (Mourente & Rodriguez, 1991) and Pleoticus muelleri (Jeckel et al., 1989).

The significance of lipids in ovarian development in P. chinensis is indicated by the increase in ovarian lipids during maturation (Table 3.2). The concentration of ovarian lipids increased from 13% in immature ovaries to 39% in mature ovaries. An increase in ovarian lipids has been reported in other shrimps, such as P. duorarum (Gehring, 1974), P. japonicus (Teshima & Kanazawa, 1983), P. aztecus, P. setiferus (Castille & Lawrence, 1989), P. monodon (Millamena & Pascual, 1990), P. kerathurus (Mourente & Rodriguez, 1991), and Pleoticus muelleri (Jeckel et al., 1989). The ovarian lipid concentrations in these shrimps increase about two folds during ovarian maturation. Since the concentration of lipids are expressed in different units among the studies, it is not easy to compare the results directly. Expressed in the

same unit as in this study, the concentrations of ovarian lipids in P. monodon and P. kerathurus increase from 6% and 12% of tissue dry weight at stage I to 16% and 25% at stage IV, respectively (Millamena & Pascual, 1990; Mourente & Rodriguez, 1991). Thus, the concentration of ovarian lipids of P. kerathurus and P. monodon are lower than that in P. chinensis.

As the total lipids increased, both neutral and polar lipids also increased during ovarian maturation in P. chinensis (Tables 3.4 & 3.5). This result is consistent with the studies in other shrimps, such as P. duorarum (Gehring, 1974), P. japonicus (Teshima & Kanazawa, 1983), P. indicus (Galois, 1984), P. kerathurus (Mourente & Rodriguez, 1991), and Pleoticus muelleri (Jeckel et al., 1989). The concentration of polar lipids was less than that of neutral lipids in P. chinensis. The ratio of neutral lipid to polar lipid (NL/PL) ranged from 1.6 to 3.4. This finding agrees with the results in P. japonicus (Teshima & Kanazawa, 1983) and the polar shrimp, Chorismus antarcticus (Clarke, 1977). The NL/PL ratio of P. japonicus ranges from 1 to 1.6 and that of Chorismus antarcticus is 1.1. However, the result of P. chinensis is in contrast to the findings in P. duorarum (Gehring, 1974), P. indicus (Galois, 1984), P. kerathurus (Mourente & Rodriguez, 1991) and Pleoticus muelleri (Jeckel et al., 1989). The NL/PL ratios are reported to be 0.21 to 0.34 in P. duorarum, to be 0.25 to 0.50 in P.

indicus, to be 0.44 to 0.97 in P. kerathurus, and to be 0.3 to 0.5 in Pleoticus muelleri.

Triglycerides, free fatty acids and cholesterol, which constituted more than 90% of neutral lipids, were the dominant neutral lipids in P. chinensis. Accumulation of triglycerides may indicate the necessarity of this compound to be stored as energy reserves in the eggs. The early larval development of shrimp larvae are largely dependent upon endogenous energy reserves (Fraser, 1989). Much of the endogenous energy reserves is in the form of triglycerides, the major depot or storage in animal cells. The reserves of triglycerides in eggs would be utilized during embryonic and naupliar development until the shrimp larvae start feeding in the protozoeal stage.

The concentration of free fatty acids in the ovary varied between 20 to 30% of neutral lipids. Levels of free fatty acids in the ovary has been reported to be 1.8% (Guary et al., 1974) and 6 to 20% of total lipids (Teshima & Kanazawa, 1983) in P. japonicus, and 5.5 to 9.5 % of total lipids in Pleoticus muelleri (Jeckel et al., 1989). Such high levels of free fatty acids in the ovary may indicate free fatty acids are important intermediates of lipid metabolism in the ovary.

As in the crab, Pachygrapsus marmoratus (Lautier & Lagarrigue, 1988), cholesterol increased to a greater extent free fatty acids in the ovary of Penaeus

chinensis. The concentration of cholesterol increased three folds while the concentrations of triglycerides and free fatty acids increased two folds only. A large amount of cholesterol accumulated in the ovary may be necessary for membrane production.

Phosphatidylcholine and phosphatidylethanolamine, which constituted more than 50% of polar lipids at stage IV, predominated in polar lipids of P. chinensis. Phosphatidylcholine is mostly responsible for the changes of ovarian polar lipids. These phospholipids may function in cellular membrane and lipid storage in oocytes as lipoproteins.

All fatty acids in the ovary increase during ovarian maturation of P. chinensis (Table 3.6). This result is similar to the result in P. kerathurus (Mourente & Rodriguez, 1991). Ovaries of Cancer magister (Allen, 1972) and Penaeus japonicus (Guary et al., 1974) contain more palmitic (16:0), palmitoleic (16:1), and oleic (18:1) acids than the other organs and tissues. In the present study, the proportions of these acids are higher in the ovary than in the hepatopancreas at mature stage (Tables 3.6b & 3.11b). The large amount of these acids in mature ovary may be due to the fact that these fatty acids are necessary for triglyceride production during vitellogenesis.

It has been suggested that polyunsaturated fatty

acids are involved in some capacity in the reproductive process of penaeids (Brown et al., 1979; Lawrence et al., 1980). A large reduction of polyunsaturated fatty acids in the ovary from stage IV to V suggests the spawned eggs contain a large amount of these acids. Clarke (1977) noted that polyunsaturated fatty acids comprised 45% of total fatty acids in egg polar lipids and 47% of total fatty acids in egg triglycerides of polar shrimp, Chorismus antarcticus. In the eggs of the crab, Pachygrapsus marmoratus, polyunsaturated fatty acids also constitute about 30% of total fatty acids (Lautier & Lagarrigue, 1988). These acids be used as components of cell membrane of the eggs.

3.4.2 Variation of lipids in hepatopancreas during ovarian maturation

The hepatopancreatic lipid concentrations appeared to be higher in stages I and II than in stages III and IV, although the differences are not statistically significant. A reduction in hepatopancreatic lipids during ovarian maturation has been reported in other penaeids, including P. japonicus (Teshima & Kanazawa, 1983) and P. kerathurus (Mourente & Rodriguez, 1991). The concentration of hepatopancreatic lipid of P. chinensis is about 10% lower than that of P. kerathurus (Mourente & Rodriguez, 1991) but about 50% higher than in

P. monodon (Millamena & Pascual, 1990). The difference in lipid concentrations among the shrimps may be due to different diets ingested by the species.

Both neutral and polar lipids in the hepatopancreas decreased from early mature stage (Stage II) to mature stage (Stage IV) in P. chinensis (Tables 3.9 & 3.10). Similar results have been reported in P. japonicus (Teshima & Kanazawa, 1983), P. monodon (Millamena & Pascual, 1990) and P. kerathurus (Mourente & Rodriguez, 1991). The concentrations of neutral lipids in P. chinensis are 30 to 50% lower than that in P. kerathurus (Mourente & Rodriguez, 1991), while the concentration of polar lipids of P. chinensis is 20 to 50% higher than those in P. kerathurus during ovarian maturation.

In P. chinensis, triglycerides, free fatty acids and cholesterol constituted more than 90% of neutral lipids in the hepatopancreas. Triglycerides alone comprised more than 60% of neutral lipids. Large amount of free fatty acids of 12 to 25% of total lipids during ovarian maturation was found in the hepatopancreas of P. chinensis. This result may be due to the fact that free fatty acids are common intermediates of metabolism of acylglycerides and phospholipids. Owing to incomplete spawning, nutrients, including lipids, carbohydrates and protein, in the residual eggs might be reabsorbed to the hepatopancreas. This reabsorption may accounted for the increase in concentrations of triglycerides and free

fatty acids in the hepatopancreas after spawning.

The concentration of hepatopancreatic cholesterol increased in P. chinensis during ovarian maturation. The values are similar to the concentrations in P. kerathurus (Mourente & Rodriguez, 1991). The hepatopancreas has been suggested to be the principal site of cholesterol absorption and storage. Since decapod crustaceans are unable to synthesize the sterol (Teshima, 1982), cholesterol accumulated in the hepatopancreas would originate exclusively from the diet. After absorbed in the hepatopancreas, cholesterol is transported to other tissues by lipoproteins, which are rich in polar lipids (Guary & Kanazawa, 1973).

The decrease of total polar lipids in the hepatopancreas of P. chinensis was mainly due to the reduction in concentration of phosphatidylcholine. This decrease of total polar lipids may be related to the transport of lipids from the hepatopancreas to the ovary (see section 3.4.4). As a whole, triglycerides and phospholipids are responsible for the changes in hepatopancreatic lipids during ovarian maturation.

The concentrations of saturated and monounsaturated fatty acids in the hepatopancreas of P. chinensis decreased during ovarian maturation (Table 3.11). Since the saturated and monounsaturated fatty acids are likely to be incorporated in triglycerides, their reduction in the hepatopancreas may be related to the decrease in

hepatopancreatic triglycerides in later mature stage.

Since crustaceans are incapable of de novo synthesis of polyunsaturated fatty acids and are exclusively dependent upon exogenous sources for these acids (D'Abramo & Lovell, 1991), the increase of polyunsaturated fatty acids in the hepatopancreas may result from feeding on food items which possesses higher contents of polyunsaturated fatty acids. Middleditch et al. (1980a) suggested that polyunsaturated fatty acids, especially eicosapentaenoic (20:5ω3) and docosahexaenoic (22:6ω3) acids, act as precursors of prostaglandins which may induce ovarian maturation. The increase in concentrations of arachidonic (20:4ω6), eicosapentaenoic (20:5ω3), and docosahexaenoic (22:6ω3) acids in polar lipids of the hepatopancreas as shown in the present study may thus be related to production of prostaglandins, as well as phospholipids which are used for lipid transport in the haemolymph.

3.4.3 Variation of lipids in muscle during ovarian maturation

The muscular lipids, which comprised about 5% of tissue dry weight, are much lower in concentration than lipids in the ovary and hepatopancreas of P. chinensis (Table 3.2). The lipid concentrations in muscle remained relatively constant during ovarian maturation.

The concentrations of both neutral and polar lipids

in muscle were much lower than those in ovary and hepatopancreas (Tables 3.14a & 3.15a). The changes in concentrations of both neutral and polar lipids were much smaller than those in ovary and hepatopancreas.

In total lipids and neutral lipids, the concentrations of fatty acids remained relatively constant (Tables 3.16a & 3.17a). There was a notable decrease of concentrations of fatty acids in polar lipids (Table 3.18a). These fatty acids may be mobilized to meet the demand of polar lipids in the ovary.

3.4.4 Mobilization of lipids during ovarian maturation

During ovarian maturation, the concentration of total lipids in ovary of Penaeus chinensis increased two folds while the concentration of lipids in hepatopancreas appeared to decrease. These results indicated that there was a mobilization of lipids from the hepatopancreas to the ovary during ovarian maturation. Mobilization of the lipids during ovarian maturation have been documented in other decapod crustaceans, such as the shrimps, Parapenaeopsis hardwickii (Kulkarni & Nagabhushanam, 1979), Penaeus indicus (Galois, 1984), P. japonicus (Teshima & Kanazawa, 1983), P. monodon (Millamena & Pascual, 1990), and P. kerathurus (Mourente & Rodriguez,

1991), and the crabs, Paratelphusa hydrodromous (Adiyodi & Adiyodi, 1971), Carcinus maenas (Paulus & Laufer, 1982), and Pachygrapsus marmoratus (Lautier & Lagarrigue, 1988). The lipids stored in the hepatopancreas are transported to the ovary in order to fulfil the great energy demand of the ovary during ovarian maturation.

The absolute amount of ovarian lipids about 1300 mg per shrimp from stage I to IV (Table 3.3). This result is close to the increment of ovarian lipids found in P. japonicus (Teshima & Kanazawa, 1983). During ovarian maturation, the absolute amount of hepatopancreatic lipids decreased about 0.36 g per shrimp (Table 3.3). However, the amount of decrease in hepatopancreatic lipids accounts for only 27% of the increment of ovarian lipids in P. chinensis. Thus, lipid requirement of the ovary does not depend solely on the lipid storage in the hepatopancreas. The hepatopancreas is regarded as a metabolic centre converting incoming lipids, rather than simply acting as a reservoir of lipids (Clarke, 1982). As Harrison (1991) stated, the strong requirement of lipids during breeding may rely on immediate ingestion of dietary lipids, in addition to stored lipids.

Concentrations of lipid classes in the ovary and hepatopancreas changed in a similar way as total lipids during ovarian maturation of P. chinensis. From the immature stage to mature stage, the concentrations of neutral lipids increased two folds in ovary while those

in the hepatopancreas decreased about 50%. Triglycerides and free fatty acids were responsible for the changes of neutral lipids in these two organs during maturation. Similar results have also reported in other penaeids (Teshima & Kanazawa, 1983; Galois, 1984; Mourente & Rodriguez, 1991). Mobilization of triglycerides and cholesterol may indicate the importance of these substances during ovarian maturation. Triglycerides are necessary to be stored in eggs for energy reserves during embryonic and pre-feeding larval development.

The concentration of polar lipids of P. chinensis exhibited a simultaneous increase in ovary and decrease in hepatopancreas during ovarian maturation. This was particularly obvious in phosphatidylcholine, a dominant component of polar lipids. This phenomenon agrees the results in P. japonicus (Teshima & Kanazawa, 1983) and P. kerathurus (Mourente & Rodriguez, 1991). Polar lipids are essential in the eggs because they are the components of cell membrane and vitellin, the major constituent of the egg yolk.

The increase in concentrations of saturated and monounsaturated fatty acids in ovary with a concurrent decrease of these acids in hepatopancreas was also found during ovarian maturation in P. chinensis. These results suggested the possible movement of fatty acids from hepatopancreas to ovary during maturation. The mobilization of fatty acids have been reported in the

shrimps, such as P. japonicus (Teshima & Kanazawa, 1983) and P. kerathurus (Mourente & Rodriguez, 1991), and the crabs, such as Paratelphusa hydrodromous (Adiyodi & Adiyodi, 1971) and Pachygrapsus marmoratus (Lautier & Lagarrigue, 1988). The variation of these acids may be related to changes of triglycerides and polar lipids during ovarian maturation because these acids are always incorporated into triglycerides and polar lipids.

In this study, the changes of lipid concentrations in ovary and hepatopancreas during maturation suggest the occurrence of mobilization of lipids from hepatopancreas to ovary. This mobilization during ovarian maturation was confirmed by a large increase of total lipids in haemolymph in the shrimp Penaeus japonicus (Teshima & Kanazawa, 1983) and the crab Pachygrapsus marmoratus (Lautier & Lagarrigue, 1988). Lipids in crustaceans are transported by lipoproteins, which are rich in polar lipids. Cholesterol in hepatopancreas is associated and thereby transported by lipoproteins to ovary (Teshima & Kanazawa, 1980; Chang & O'Connor, 1983). Triglycerides are converted into polar lipids in the hepatopancreas for export into the haemolymph as components of high density lipoproteins (HDL) (Harrison, 1990). Afterwards, the lipoproteins are rapidly transported from the haemolymph to the ovary and the components of lipoproteins are released inside the ovary. Part of polar lipids is reconverted to triglycerides. These triglycerides are

either be used for energy production during the manufacture of oocytes, or be stored as fat droplets in oocytes. The appearance of lipid globules has been reported in the ovary of shrimps, such as Crangon crangon, several species of Palaemonetes (Beam & Kessel, 1963), Penaeus japonicus (Yano, 1988), P. monodon (Tan-Fermin & Pudadera, 1989) and the crayfish Orconestes species (Beam & Kessel, 1963). Part of polar lipids, as well as cholesterol is used for membrane construction as size and number of the oocytes increase during ovarian maturation. Certain amount of polar lipids is also present in the yolk.

Chapter 4 Variation of lipid composition during ovarian maturation of Metapenaeus ensis

4.1 Introduction

This chapter presents the variation of lipids composition in ovary, hepatopancreas and muscle during ovarian maturation in Metapenaeus ensis.

4.2 Materials and methods

4.2.1 Experimental animals

Wild adult female Metapenaeus ensis were acquired from the fish market at Lee Yuen Mun. Females were transported alive to the laboratory. Shrimps at different stages of ovarian maturation were separated. Five stages could be identified, based on a scheme modified from Yano (1985).

Stage I (Immature stage):

The ovary was translucent, unpigmented with no distinguishable outline. The gonadosomatic index (GSI) value was within 0.49 and 1.27%.

Stage II (Developing stage):

The ovary was visible as a thick opaque line along the dorsal central axis. The GSI value was within 1.51 and 2.05%.

Stage III (Slightly mature):

The ovary was visible through the exoskeleton and yellow in colour. The GSI value was within 2.28 and 3.78%.

Stage IV (Mature stage):

The ovarian lobes were larger than in the preceding stage and clearly visible through the exoskeleton. The colour of ovary changed to green. The GSI value was within 4.05 and 8.88%.

Stage V (Spent ovary stage):

Shrimp just after spawning were used.

Treatment of the shrimp and tissue samples were the same as described in section 3.2.1.

4.2.2 Total lipid extraction and quantification

Experimental details were the same as described in section 3.2.2

4.2.3 Separation and quantification of lipid classes

The samples of each maturation stage were pooled together. Concentrations of lipid classes were determined in triplicate. Experimental details were the same as described in section 3.2.3.

4.2.4 Fatty acid analysis

The pooled tissue samples were analyzed in triplicate. Experimental details were the same as described in section 3.2.4.

4.3 Results

4.3.1 Biometric data

The carapace length and body weight Metapenaeus ensis studied in each maturation stage are shown in Table 4.1. The carapace length of shrimps at different maturation stages are similar. The wet and dry body weights were the highest at stage IV. The gonadosomatic index (GSI) increased from stage I to IV and dropped in spent ovaries. The hepatosomatic index (HSI) varied in a similar pattern but the differences among the stages are not statistically significant.

4.3.2 Variation of total lipids

The concentration of total lipids in the ovary increased two folds from stage I to IV, and decreased greatly in spent ovaries (Table 4.2). The concentration of hepatopancreatic lipids dropped from 40% of tissue dry weight at stage I to less than 20% at stage IV. The concentration of muscular lipids was lower than 5% of tissue dry weight, and remained roughly constant during ovarian maturation.

It can be estimated that the absolute amount of ovarian lipids increased from 13 mg per shrimp at stage I to 291 mg at stage IV, and then declined to 25 mg in spent

ovaries (Table 4.3). At the same period, hepatopancreatic lipids decreased from 127 mg per shrimp at stage I to 85 mg at stage IV, and dropped to 59 mg at stage V.

4.3.3 Variation of lipids in ovary

4.3.3.1 Neutral lipid classes

The concentration of total neutral lipids in the ovary increased three folds from stage I to IV (Table 4.4), and dropped to a low level in spent ovaries. Triglycerides, cholesterol and free fatty acids were dominant classes of neutral lipids in the ovary, often comprising 90% of neutral lipids. The concentrations of triglycerides and cholesterol increased from 21 mg/g and 16 mg/g tissue dry weight at stage I to 112 mg/g and 146 mg/g at stage IV, and then decreased to 51 mg/g and 96 mg/g in spent ovaries, respectively. The concentration of free fatty acids increased about two folds from stage I to stage IV. The concentrations of diglycerides and monoglycerides were about 1 mg/g tissue dry weight or low. The concentrations of these two acylglycerides decreased from stage I to IV.

4.3.3.2 Polar lipid classes

The concentration of ovarian polar lipids increased four folds from stage I to IV, and decreased by 62% in stage V (Table 4.5). All polar lipid classes increased

their concentrations during ovarian maturation. Marked increases were found in phosphatidylcholine and phosphatidylethanolamine; their concentrations increased from 13 mg/g and 14 mg/g tissue dry weight at stage I to 90 mg/g and 44 mg/g at stage IV, and decreased to 24 mg/g and 20 mg/g in spent ovaries, respectively. From stage I to IV, the concentration of phosphatidylserine increased about two folds.

4.3.3.3 Fatty acid composition

Saturated, monounsaturated and polyunsaturated fatty acids in total lipids in the ovary increased about two folds during maturation (Table 4.6a). Their concentrations decreased in spent ovaries. The predominant fatty acids in ovarian lipids were palmitic (16:0), stearic (18:0), palmitoleic (16:1) and oleic (18:1) acids (Table 4.6b). They comprised about 70% of total fatty acids and increased during ovarian maturation. Arachidonic (20:4ω6), eicosapentaenoic (20:5ω3), docosapentaenoic (22:5ω3) and docosahexaenoic (22:6ω3) acids, the dominated in polyunsaturated fatty acids of the mature ovary, comprised about 70% of total polyunsaturated fatty acids. The concentrations of eicosapentaenoic and docosahexaenoic acids increased from 1.6 mg/g and 0.2 mg/g of tissue dry weight at stage I to 6.0 mg/g and 1.3 mg/g at stage IV, respectively. The concentrations of palmitic, stearic,

oleic and arachidonic acids also increased about two folds from stage I to stage IV.

Similar composition of fatty acids and similar patterns of changes were found in ovarian polar and neutral lipids (Tables 4.7 & 4.8). The concentrations of saturated, monounsaturated and polyunsaturated fatty acids in both polar and neutral lipids decreased in spent ovaries.

4.3.4 Variation of lipids in hepatopancreas

4.3.4.1 Neutral lipid classes

The concentration of total neutral lipids in the hepatopancreas at stage IV was half of the value at stage I (Table 4.9). The value increased by 23% from stage IV to V. Triglycerides, free fatty acids and cholesterol constituted more than 90% of neutral lipids in the hepatopancreas. The concentrations of triglycerides and cholesterol decreased from 186 mg/g and 51 mg/g of tissue dry weight at stage I to 50 mg/g and 25 mg/g at stage IV, respectively. The concentration of free fatty acids increased from 54 mg/g of tissue dry weight at stage I to 84 mg/g at stage II, and then decreased to 58 mg/g in stage V. Small amounts of diglycerides and monoglycerides of less than 2 mg/g tissue dry weight, decreased during ovarian maturation.

4.3.4.2 Polar lipid classes

The concentration of total polar lipids in the hepatopancreas appeared to decrease from stage I to a low level at stage II (Table 4.10). The concentration then remained roughly constant towards the later stages. The dominant classes were phosphatidylcholine and phosphatidylethanolamine, which together comprised more than 60% of polar lipids. The concentrations of phosphatidylcholine and phosphatidylethanolamine decreased from 61 mg/g and 33 mg/g of tissue dry weight at stage I to 23 mg/g and 19 mg/g at stage IV, respectively. The concentrations of sphingomyelin and phosphatidylserine remained relatively constant during ovarian maturation.

4.3.4.3 Fatty acid composition

Palmitic (16:0), stearic (18:0), palmitoleic (16:1), oleic (18:1), and eicosapentaenoic (20:5ω3) acids were dominant fatty acids in hepatopancreatic lipids at stage IV, comprising about 70% of total fatty acids (Table 4.11b). The concentrations of saturated and fatty acids decreased 25% from stage I to IV while the concentration of polyunsaturated fatty acids increased by about 40% (Table 4.11a). There was no obvious trend of the concentration of monounsaturated fatty acids during ovarian maturation. From stage IV to V, the concentrations of saturated and

monounsaturated acids increased about 40% while polyunsaturated fatty acids decreased about 10%. The hepatopancreatic lipids contained higher concentrations of eicosapentaenoic (20:5ω3) and docosahexaenoic (22:6ω3) acids than the ovarian lipids in every stage of ovarian maturation (Tables 6a & 24a).

Similar fatty acid composition and similar patterns of changes of fatty acids were found in both neutral and polar lipids (Tables 4.12 & 4.13).

4.3.5 Variation of lipids in muscle

4.3.5.1 Neutral lipid classes

The concentration of total neutral lipids in muscle was below 100 mg/g tissue dry weight and remained roughly constant during ovarian maturation (Table 4.14). The major classes in the muscle were triglycerides, cholesterol and free fatty acids. Small amounts of diglycerides and monoglycerides, which were less than 1 mg/g tissue dry weight, were present in all stages. Their concentrations remained relatively constant during ovarian maturation.

4.3.5.2 Polar lipid classes

The concentration of total polar lipids constituted below 25 mg/g tissue dry weight and did not vary during

ovarian maturation (Table 4.15). Similarly, all polar lipid classes remained roughly constant during ovarian maturation.

4.3.5.3 Fatty acid composition

Palmitic (16:0), stearic (18:0), oleic (18:1), eicosapentaenoic (20:5ω3) and docosahexaenoic (22:6ω3) acids were the major fatty acids in muscular lipids (Table 4.16b). During ovarian maturation, the concentrations of fatty acids in total lipids, neutral lipids and polar lipids did not changed markedly (Tables 4.16a & 4.17a). Higher proportion of polyunsaturated fatty acids was found in muscular polar lipids than neutral lipids while higher proportion of saturated fatty acids were found in neutral lipids than in polar lipids during ovarian maturation (Tables 4.17b & 4.18b).

Table 4.1 Biometric data for female Metapenaeus ensis at different ovarian maturation stages.

	Ovarian maturation stage				
	I	II	III	IV	V
Carapace length (cm)	3.8±0.1	4.0±0.2	3.9±0.2	4.2±0.1	3.7±0.2
Total body weight					
wet (g)	28±2	33±4	31±4	36±1	29±4
dry (g)*	7.4±0.5	8.3±1.6	7.2±0.8	9.5±0.4	6.6±0.7
Gonadosomatic index, GSI (%) *	0.8±0.2	1.5±0.1	2.8±0.2	7.4±0.5	1.5±0.5
Hepatosomatic index, HSI (%)	4.2±0.4	4.5±0.3	4.3±0.1	4.6±0.2	4.1±0.4
No. of shrimp	6	6	8	12	7

Values are means ± SE.

* Values are significantly different (1-way ANOVA; $P < 0.05$).

Table 4.2 Total lipid concentrations (% of tissue dry weight) in the ovary, hepatopancreas and muscle during ovarian maturation of Metapenaeus ensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Ovary *	21.9±3.6	22.4±3.0	28.3±2.8	43.2±3.2	26.4±2.8
Hepatopancreas *	39.5±1.1	34.8±1.6	21.3±1.4	19.4±1.9	18.1±3.1
Muscle	4.4±1.0	4.3±0.8	4.9±0.9	4.7±0.6	4.7±0.4

Values are means ± SE (n = 6 to 12).

* Values are significantly different (1-way ANOVA, $P < 0.05$).

Table 4.3 Absolute amounts of lipid (mg) per shrimp in the ovary, hepatopancreas and muscle during ovarian maturation of Metapenaeus ensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Ovary*	13±2	28±6	58±10	291±31	25±1
Hepatopancreas*	127±21	132±16	72±12	85±8	59±11

Values are means ± SE (n = 6 to 12).

* Values are significantly different (1-way, ANOVA, $P < 0.05$).

Table 4.4 Concentrations of neutral lipid classes (mg lipid per g tissue dry weight) in ovary of Metapenaeus ensis during ovarian maturation.

	Ovarian maturation stage				
	I	II	III	IV	V
Monoglycerides*	1.14±0.08	0.84±0.03	0.43±0.04	0.34±0.11	0.35±0.06
Diglycerides*	0.81±0.09	0.15±0.04	0.28±0.02	0.14±0.03	0.16±0.04
Triglycerides*	21±4	19±2	114±9	112±4	51±5
Cholesterol*	32±4	66±3	112±6	146±12	96±12
Free fatty acids*	16±1	24±3	28±5	39±6	11±1
Total neutral lipids*	72±9	111±8	254±12	297±30	159±18

Values are means ± SE of tripilcate determinations.

The concentration of total neutral lipids is the sum of the concentrations of neutral lipid classes.

* Values are significantly different (1-way ANOVA; $P < 0.05$).

Table 4.5 Concentrations of polar lipid classes (mg lipid per g tissue dry weight) in ovary of Metapenaeus ensis during ovarian maturation.

	Ovarian maturation stage				
	I	II	III	IV	V
Sphingomyelin	15.4±1.8	14.2±5.1	16.2±2.5	22.8±0.9	21.6±2.0
Phosphatidylcholine*	12.6±2.4	27.2±4.7	92.1±2.1	89.6±2.5	24.4±4.2
Phosphatidylserine*	9.8±1.4	21.6±2.4	23.2±1.1	21.3±2.7	8.4±2.6
Phosphatidylethanolamine*	14.0±1.4	20.5±0.8	59.2±1.1	43.8±2.5	20.0±4.0
Total polar lipids*	57±4	87±8	183±3	204±10	78±9

Values are means ± SE of triplicate determinations.

The concentration of total polar lipids are determined together with neutral lipids listed in Table 3.4 after separation of lipid classes.

* Values are significantly different (1-way ANOVA; $P < 0.05$).

Table 4.6a Concentrations of fatty acid content in total ovarian lipids during ovarian maturation of Metapenaeus ensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0*	0.63±0.01	0.77±0.07	0.62±0.08	0.79±0.02	0.19±0.03
16:0*	7.41±0.15	8.75±0.34	11.4±0.4	16.4±0.5	6.51±0.17
18:0*	5.84±0.25	7.85±0.32	8.52±0.39	8.57±0.26	5.27±0.07
20:0*	0.15±0.01	0.37±0.05	0.68±0.08	0.72±0.11	0.24±0.03
Total*	14.0±0.4	17.7±0.8	21.3±0.9	26.5±0.9	12.2±0.2
Monounsaturated					
16:1*	5.32±0.18	7.14±0.45	13.2±0.18	16.0±0.80	8.62±0.20
18:1*	6.95±0.24	10.4±0.1	15.3±0.2	12.4±0.8	9.87±0.44
20:1*	0.60±0.02	0.69±0.06	0.41±0.06	0.33±0.02	0.67±0.04
Total*	12.9±0.6	18.2±0.6	28.9±0.6	28.7±1.6	19.2±0.7
PUFA (ω6)**					
18:2	0.59±0.03	0.46±0.05	0.64±0.10	0.57±0.01	0.42±0.05
20:2*	0.84±0.08	0.94±0.04	0.61±0.02	0.52±0.04	0.43±0.06
20:4*	0.79±0.06	1.62±0.18	1.69±0.25	1.61±0.14	0.53±0.02
PUFA (ω3)***					
18:3	0.97±0.09	0.78±0.11	0.58±0.08	0.63±0.11	0.91±0.10
18:4*	0.18±0.04	0.14±0.06	0.47±0.03	0.18±0.02	0.17±0.02
20:5*	1.58±0.13	2.47±0.58	4.06±0.57	6.03±0.12	0.64±0.03
22:5*	0.78±0.08	0.74±0.11	0.92±0.06	0.80±0.02	0.48±0.03
22:6*	0.20±0.06	0.63±0.08	1.22±0.08	1.28±0.16	0.87±0.07
Total PUFA*	5.9±0.6	8.8±0.8	10.2±0.9	11.6±0.4	4.0±0.4
Total*	32.8±1.5	44.8±2.5	60.4±2.4	66.8±2.0	35.8±0.9

Data are means ± SE of triplicate determinations, and expressed as mg fatty acid/g tissue dry weight.

* Values are significantly different (1-way ANOVA; $P < 0.05$).

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

Table 4.6b Relative amounts of total ovarian fatty acid content during ovarian maturation of Metapenaeus ensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0	1.9%	1.7%	1.0%	1.2%	0.50%
16:0	23.6%	19.6%	18.9%	24.5%	18.2%
18:0	17.8%	17.5%	14.1%	12.8%	14.7%
20:0	0.5%	0.8%	1.1%	1.1%	0.7%
Total	43%	40%	35%	40%	34%
Monounsaturated					
16:1	16.2%	16.0%	21.9%	24.0%	24.1%
18:1	21.2%	23.2%	25.3%	18.5%	27.6%
20:1	1.8%	1.5%	0.7%	0.5%	1.9%
Total	39%	41%	48%	43%	54%
PUFA (ω6)**					
18:2	1.8%	1.0%	1.1%	0.9%	1.2%
20:2	2.6%	2.1%	1.0%	0.8%	1.2%
20:4	2.4%	3.6%	2.8%	2.4%	1.5%
PUFA (ω3)***					
18:3	3.0%	1.7%	1.0%	0.9%	1.2%
18:4	2.6%	2.1%	1.0%	0.8%	0.5%
20:5	4.8%	7.8%	6.7%	9.0%	1.8%
22:5	2.4%	1.7%	1.5%	1.2%	1.3%
22:6	0.6%	1.4%	2.0%	1.9%	2.4%
Total PUFA	18%	19%	17%	17%	12%

Data are means and expressed as % of total fatty acids.

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

Table 4.7a Concentrations of fatty acid content in ovarian neutral lipids during ovarian maturation of Metapenaeus ensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0*	0.32±0.02	0.46±0.03	0.56±0.05	0.48±0.06	0.31±0.03
16:0*	5.93±0.19	5.25±0.14	5.72±0.33	10.0±0.3	3.84±0.06
18:0*	4.67±0.28	4.71±0.15	4.26±0.23	5.23±0.08	3.11±0.16
20:0*	0.12±0.04	0.22±0.03	0.34±0.03	0.44±0.03	0.24±0.03
Total*	11.0±0.3	10.6±0.3	10.9±0.6	16.2±0.5	7.5±0.2
Monounsaturated					
16:1*	5.26±0.18	5.28±0.21	7.61±0.18	8.77±0.22	6.09±0.06
18:1*	4.56±0.23	5.24±0.16	6.65±0.24	6.54±0.12	4.82±0.16
20:1*	0.38±0.02	0.41±0.02	0.21±0.02	0.34±0.02	0.40±0.02
Total*	10.2±0.4	10.9±0.4	14.5±0.4	15.7±0.3	11.3±0.2
PUFA (ω6)**					
18:2*	0.47±0.02	0.28±0.06	0.32±0.05	0.35±0.03	0.44±0.03
20:2	0.67±0.02	0.56±0.02	0.31±0.01	0.32±0.02	0.13±0.03
20:4*	0.53±0.03	0.97±0.17	0.85±0.04	0.98±0.05	0.41±0.01
PUFA (ω3)***					
18:3	0.28±0.02	0.47±0.04	0.29±0.02	0.38±0.01	0.44±0.04
18:4*	0.14±0.04	0.08±0.02	0.24±0.02	0.11±0.01	0.12±0.03
20:5*	1.06±0.10	2.08±0.20	2.03±0.15	3.48±0.14	0.83±0.05
22:5*	0.62±0.06	0.44±0.04	0.46±0.02	0.49±0.02	0.28±0.05
22:6*	0.14±0.01	0.28±0.06	0.39±0.03	0.48±0.02	0.24±0.01
Total PUFA*	3.9±0.3	5.2±0.6	4.9±0.3	6.6±0.2	2.7±0.3
Total*	25.2±1.1	26.8±1.3	30.2±1.4	38.4±1.0	21.5±0.8

Data are means ± SE of triplicate determinations, and expressed as mg fatty acid/g tissue dry weight.

* Values are significantly different (1-way ANOVA; $P < 0.05$).

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

Table 4.7b Relative amounts of fatty acid content in ovarian neutral lipids during ovarian maturation of Metapenaeus ensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0	1.3%	1.7%	1.9%	1.3%	1.4%
16:0	23.6%	19.6%	18.9%	26.0%	17.9%
18:0	18.6%	17.6%	14.1%	13.6%	14.5%
20:0	0.5%	0.8%	1.1%	1.1%	1.1%
Total	44%	40%	36%	42%	35%
Monounsaturated					
16:1	20.9%	19.7%	25.2%	22.8%	28.3%
18:1	18.1%	19.6%	22.0%	17.0%	22.4%
20:1	1.5%	1.5%	0.7%	0.9%	1.8%
Total	41%	41%	48%	41%	52%
PUFA (ω6)**					
18:2	1.9%	1.0%	1.1%	0.9%	1.1%
20:2	2.7%	2.1%	1.0%	0.8%	0.6%
20:4	2.1%	3.6%	2.8%	2.6%	1.9%
PUFA (ω3)***					
18:3	1.1%	1.7%	1.0%	1.0%	2.0%
18:4	0.6%	0.3%	0.8%	0.3%	0.6%
20:5	4.2%	7.8%	6.7%	9.1%	3.9%
22:5	2.5%	1.7%	1.5%	1.3%	1.3%
22:6	0.6%	1.0%	1.3%	1.3%	1.1%
Total PUFA	15%	19%	16%	17%	13%

Data are means and expressed as % of total fatty acids.

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

Table 4.8a Concentrations of fatty acid content in ovarian polar lipids during ovarian maturation of Metapenaeus ensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0*	0.40±0.05	0.30±0.09	0.58±0.05	0.24±0.09	0.26±0.03
16:0*	1.19±0.11	3.41±0.22	5.14±0.11	4.92±0.21	1.95±0.04
18:0*	0.93±0.22	3.06±0.19	3.83±0.18	2.57±0.15	1.58±0.11
20:0*	0.02±0.01	0.14±0.03	0.31±0.03	0.22±0.03	0.07±0.02
Total*	2.5±0.4	6.9±0.4	9.9±0.4	7.9±0.5	3.9±0.2
Monounsaturated					
16:1*	0.85±0.07	2.78±0.26	5.94±0.38	4.81±0.10	2.59±0.19
18:1*	1.11±0.16	4.06±0.31	6.89±0.23	3.71±0.35	2.96±0.06
20:1	0.10±0.03	0.27±0.07	0.18±0.05	0.10±0.02	0.20±0.04
Total*	2.1±0.3	7.1±0.6	13.0±0.7	8.6±0.5	5.8±0.9
PUFA (ω6)**					
18:2*	0.09±0.03	0.18±0.02	0.29±0.02	0.19±0.03	0.27±0.01
20:2*	0.23±0.05	0.37±0.03	0.27±0.03	0.56±0.02	0.24±0.03
20:4*	0.13±0.03	0.63±0.03	0.76±0.02	0.63±0.01	0.16±0.03
PUFA (ω3)***					
18:3*	0.16±0.05	0.30±0.03	0.27±0.01	0.19±0.04	0.27±0.02
18:4	0.03±0.02	0.05±0.02	0.07±0.03	0.05±0.02	0.04±0.02
20:5*	0.35±0.03	1.45±0.18	1.93±0.15	1.91±0.16	0.39±0.03
22:5*	0.12±0.04	0.29±0.03	0.41±0.16	0.64±0.05	0.22±0.04
22:6*	0.13±0.03	0.23±0.01	0.35±0.05	0.38±0.03	0.26±0.03
Total PUFA*	1.2±0.3	3.5±0.3	4.4±0.4	4.5±0.4	1.7±0.2
Total*	5.8±0.9	17.5±1.4	27.3±1.4	21.1±1.3	11.3±1.3

Data are means ± SE of triplicate determinations, and expressed as mg fatty acid/g tissue dry weight.

* Values are significantly different (1-way ANOVA; $P < 0.05$).

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

Table 4.8b Relative amounts of fatty acid content in ovarian polar lipids during ovarian maturation of Metapenaeus ensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0	6.8%	1.7%	2.1%	1.1%	2.3%
16:0	20.3%	19.5%	18.9%	23.3%	17.2%
18:0	16.0%	17.5%	14.1%	12.2%	14.0%
20:0	0.4%	0.8%	1.1%	1.0%	0.6%
Total	44%	40%	36%	38%	34%
Monounsaturated					
16:1	14.6%	15.9%	21.8%	22.8%	22.8%
18:1	19.0%	23.1%	25.3%	17.6%	26.2%
20:1	1.6%	1.5%	0.7%	0.5%	1.8%
Total	35%	40%	48%	41%	51%
PUFA (ω6)**					
18:2	1.6%	1.0%	1.1%	0.8%	1.1%
20:2	3.9%	2.1%	1.0%	3.0%	1.4%
20:4	2.2%	3.6%	2.8%	3.0%	1.4%
PUFA (ω3)***					
18:3	2.7%	1.7%	1.0%	0.9%	2.4%
18:4	0.5%	0.3%	0.3%	0.3%	0.4%
20:5	6.0%	8.3%	7.1%	9.1%	3.4%
22:5	2.1%	1.6%	1.5%	3.0%	1.9%
22:6	2.2%	1.3%	1.3%	1.8%	2.3%
Total PUFA	21%	20%	16%	21%	15%

Data are means and expressed as % of total fatty acids.

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

Table 4.9 Concentrations of neutral lipid classes (mg lipid per g tissue dry weight) in hepatopancreas of Metapenaeus ensis during ovarian maturation.

	Ovarian maturation stage				
	I	II	III	IV	V
Monoglycerides*	1.76±0.05	0.84±0.13	1.17±0.17	0.68±0.05	1.01±0.09
Diglycerides	0.57±0.23	0.38±0.11	0.26±0.05	0.36±0.04	0.26±0.02
Triglycerides*	186±17	137±20	49±5	50±5	75±10
Cholesterol*	51±6	63±5	66±9	25±5	26±1
Free fatty acids*	54±3	84±7	76±11	58±6	62±8
Total neutral lipids*	293±25	284±33	193±25	134±16	165±19

Values are means ± SE of tripilcate determinations.

The concentration of total neutral lipids is the sum of the concentrations of neutral lipid classes.

* Values are significantly different (1-way ANOVA; $P < 0.05$).

Table 4.10 Concentrations of polar lipid classes (mg lipid per g tissue dry weight) in hepatopancreas of Metapenaeus ensis during ovarian maturation.

	Ovarian maturation stage				
	I	II	III	IV	V
Sphingomyelin	16.2±0.6	14.4±2.4	14.1±2.4	18.4±0.3	12.9±0.2
Phosphatidylcholine*	60.6±1.6	42.7±1.1	22.1±1.4	23.4±1.1	23.7±0.8
Phosphatidylserine	19.3±2.5	18.8±1.0	16.0±1.3	19.7±3.1	20.3±0.5
Phosphatidylethanolamine*	32.8±2.1	18.8±1.0	22.6±0.4	19.1±0.8	19.4±0.8
Total polar lipids	112±18	60±5	65±16	66±16	68±3

Values are means ± SE of triplicate determinations.

The concentration of total polar lipids are determined together with neutral lipids listed in Table 3.4 after separation of lipid classes.

* Values are significantly different (1-way ANOVA; $P < 0.05$).

Table 4.11a Concentrations of hepatopancreatic fatty acid content during ovarian maturation of Metapenaeus ensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0*	9.09±0.15	6.17±0.21	5.81±0.10	6.34±0.20	8.18±0.21
16:0*	30.7±0.1	32.2±0.1	27.7±0.1	20.7±0.1	36.1±0.2
18:0	15.8±0.3	14.3±1.0	13.7±0.3	13.9±0.5	14.8±0.2
20:0*	0.35±0.02	0.40±0.04	0.33±0.01	0.18±0.02	0.17±0.02
Total*	55.8±0.5	53.1±1.3	47.6±0.5	41.2±0.8	59.3±0.8
Monounsaturated					
16:1*	12.9±0.1	28.9±0.1	27.1±0.1	15.8±0.1	14.8±0.3
18:1*	18.8±0.3	19.8±0.2	20.1±0.3	15.2±0.3	23.7±0.3
20:1*	6.89±0.44	3.78±0.08	1.66±0.08	2.43±0.12	6.52±0.11
Total*	38.6±0.8	52.5±0.5	48.9±0.5	33.5±0.5	45.1±0.2
PUFA (ω6)**					
18:2*	2.64±0.18	2.48±0.06	3.57±0.11	4.18±0.11	5.08±0.38
20:2*	2.58±0.10	1.59±0.08	1.89±0.09	1.07±0.11	2.85±0.11
20:4*	1.33±0.11	7.28±0.14	5.79±0.16	7.11±0.16	6.38±0.04
PUFA (ω3)***					
18:3*	3.09±0.16	4.15±0.19	5.59±0.11	3.61±0.16	1.56±0.05
18:4*	0.59±0.02	0.37±0.01	0.26±0.08	0.22±0.04	0.79±0.03
20:5*	12.7±0.1	12.5±0.1	13.4±0.2	14.3±0.3	10.6±0.3
22:5*	2.12±0.20	4.23±0.12	2.16±0.30	4.65±0.19	3.84±0.14
22:6*	5.92±0.16	4.94±0.05	7.53±0.15	8.47±0.09	6.91±0.19
Total PUFA*	31.0±2.8	37.8±0.7	40.2±1.2	43.7±1.1	38.0±1.1
Total*	125±4	143±3	137±2	118±2	142±2

Data are means ± SE of triplicate determinations, and expressed as mg fatty acid/g tissue dry weight.

* Values are significantly different (1-way ANOVA; $P < 0.05$).

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

Table 4.11b Relative amounts of total hepatopancreatic fatty acid content during ovarian maturation of Metapenaeus ensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0	7.2%	4.3%	4.3%	5.4%	5.7%
16:0	24.5%	22.5%	20.3%	17.5%	25.4%
18:0	12.6%	10.0%	10.0%	11.8%	10.4%
20:0	0.3%	0.3%	0.2%	0.2%	0.1%
Total	45%	37%	35%	35%	42%
Monounsaturated					
16:1	10.3%	20.2%	19.8%	13.4%	10.4%
18:1	15.0%	13.9%	14.7%	12.9%	16.7%
20:1	5.5%	2.6%	1.2%	2.1%	4.6%
Total	31%	37%	36%	28%	31%
PUFA (ω6)**					
18:2	2.1%	1.7%	2.6%	3.5%	3.6%
20:2	2.1%	1.1%	1.4%	0.9%	2.0%
20:4	1.1%	5.1%	4.2%	6.0%	4.5%
PUFA (ω3)***					
18:3	2.5%	2.9%	4.1%	3.1%	1.1%
18:4	0.5%	0.3%	0.2%	0.2%	0.6%
20:5	10.1%	8.8%	9.8%	12.1%	7.4%
22:5	1.7%	3.0%	1.6%	3.9%	2.7%
22:6	4.7%	3.5%	5.5%	7.2%	4.9%
Total PUFA	24%	26%	29%	37%	27%

Data are means and expressed as % of total fatty acids.

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

Table 4.12a Concentrations of fatty acid content in hepatopancreatic neutral lipids during ovarian maturation of Metapenaeus ensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0*	6.91±0.11	4.32±0.34	3.99±0.36	4.12±0.09	6.30±0.41
16:0*	23.3±0.3	22.6±0.2	18.6±0.1	13.5±0.3	27.8±0.5
18:0*	12.0±0.5	10.0±0.4	9.16±0.2	9.05±0.2	11.4±0.6
20:0*	0.27±0.03	0.28±0.02	0.22±0.03	0.22±0.02	0.21±0.02
Total*	42.5±0.8	37.2±0.9	32.0±0.8	26.9±0.6	45.7±1.3
Monounsaturated					
16:1*	9.83±0.26	20.2±0.5	18.2±0.3	10.3±0.4	11.4±0.3
18:1*	14.3±0.2	13.8±0.3	13.5±0.4	9.88±0.27	18.3±0.1
20:1*	5.24±0.12	2.65±0.25	1.48±0.08	1.58±0.13	5.02±0.32
Total*	29.3±0.6	37.2±1.0	33.1±0.8	21.8±0.7	34.7±0.8
PUFA (ω6)**					
18:2*	2.01±0.17	1.74±0.17	2.39±0.11	2.72±0.27	3.91±0.25
20:2	1.26±0.13	1.11±0.12	1.27±0.23	0.70±0.13	2.19±0.19
20:4*	1.01±0.09	5.10±0.12	3.88±0.16	4.62±0.20	4.91±0.24
PUFA (ω3)***					
18:3*	2.35±0.09	2.91±0.17	3.75±0.11	2.35±0.16	1.20±0.23
18:4*	0.45±0.02	0.26±0.07	0.20±0.04	0.14±0.01	0.61±0.04
20:5*	8.64±0.14	7.77±0.40	7.96±0.28	8.02±0.30	8.31±0.20
22:5*	1.61±0.22	2.96±0.23	2.45±0.14	3.02±0.27	2.96±0.31
22:6*	4.50±0.12	3.46±0.07	5.05±0.29	5.51±0.15	5.32±0.17
Total PUFA*	21.8±0.6	25.3±1.3	26.9±0.3	27.1±0.7	29.4±1.5
Total*	93.6±2.4	99.2±4.6	92.0±2.8	75.7±2.6	110±4

Data are means ± SE of triplicate determinations, and expressed as mg fatty acid/g tissue dry weight.

* Values are significantly different (1-way ANOVA; $P < 0.05$).

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

Table 4.12b Relative amounts of fatty acid content in hepatopancreatic neutral lipids during ovarian maturation of Metapenaeus ensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0	7.4%	4.4%	4.3%	5.4%	5.7%
16:0	24.9%	22.7%	20.2%	17.8%	25.3%
18:0	12.8%	10.1%	10.0%	12.0%	10.4%
20:0	0.3%	0.3%	0.2%	0.3%	0.2%
Total	45%	38%	35%	36%	42%
Monounsaturated					
16:1	10.5%	20.4%	19.7%	13.6%	10.4%
18:1	15.2%	14.0%	14.6%	13.1%	16.6%
20:1	5.6%	2.7%	1.6%	2.1%	4.6%
Total	31%	37%	36%	29%	32%
PUFA (ω6)**					
18:2	2.1%	1.8%	2.6%	3.6%	3.6%
20:2	1.3%	1.1%	1.4%	0.91%	2.0%
20:4	1.1%	5.1%	4.2%	6.1%	4.5%
PUFA (ω3)***					
18:3	2.5%	2.9%	4.1%	3.1%	1.1%
18:4	0.5%	0.3%	0.2%	0.2%	0.6%
20:5	9.2%	7.8%	8.7%	10.6%	7.6%
22:5	1.7%	3.0%	2.7%	4.0%	2.7%
22:6	4.8%	3.5%	5.5%	7.3%	4.8%
Total PUFA	24%	25%	29%	35%	26%

Data are means and expressed as % of total fatty acids.

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

Table 4.13a Concentrations of fatty acid content in hepatopancreatic polar lipids during ovarian maturation of Metapenaeus ensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0*	1.82±0.10	1.60±0.06	1.74±0.09	1.97±0.25	1.55±0.10
16:0*	6.14±0.04	8.37±0.15	8.32±0.14	6.42±0.22	6.86±0.31
18:0*	3.15±0.25	3.72±0.19	4.10±0.19	4.32±0.24	2.81±0.33
20:0*	0.07±0.01	0.10±0.02	0.10±0.02	0.06±0.02	0.03±0.02
Total*	11.2±0.3	13.8±0.4	14.3±0.4	12.8±0.6	11.3±0.6
Monounsaturated					
16:1*	2.59±0.12	7.50±0.20	8.13±0.38	4.91±0.16	2.82±0.28
18:1*	3.75±0.22	5.15±0.28	6.03±0.17	4.71±0.15	4.51±0.22
20:1	1.38±0.02	0.98±0.07	0.50±0.02	0.75±0.11	1.24±0.08
Total*	7.7±0.4	13.6±0.7	14.7±0.5	10.4±0.4	8.6±0.6
PUFA (ω6)**					
18:2*	0.53±0.05	0.64±0.08	1.07±0.12	1.30±0.12	0.97±0.08
20:2*	0.52±0.02	0.41±0.03	0.57±0.06	0.33±0.07	0.54±0.10
20:4*	1.27±0.08	2.89±0.24	2.74±0.18	3.20±0.18	2.31±0.21
PUFA (ω3)***					
18:3*	0.62±0.01	1.08±0.18	1.68±0.11	1.12±0.23	0.30±0.07
18:4	0.12±0.04	0.10±0.02	0.08±0.03	0.07±0.04	0.15±0.04
20:5*	2.54±0.15	3.26±0.03	4.01±0.19	4.45±0.20	2.01±0.30
22:5*	0.42±0.03	1.10±0.03	0.65±0.03	1.44±0.14	0.73±0.08
22:6*	1.18±0.05	1.28±0.05	2.26±0.12	2.63±0.20	1.31±0.07
Total PUFA*	7.2±0.2	10.8±0.4	13.1±0.5	14.5±0.6	8.3±0.6
Total*	26.1±1.0	38.2±1.7	42.0±1.7	37.7±2.1	28.1±2.6

Data are means ± SE of triplicate determinations, and expressed as mg fatty acid/g tissue dry weight.

* Values are significantly different (1-way ANOVA; $P < 0.05$).

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

Table 4.13b Relative amounts of fatty acid content in hepatopancreatic polar lipids during ovarian maturation of Metapenaeus ensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0	7.0%	4.2%	4.2%	5.2%	5.5%
16:0	23.4%	21.9%	19.8%	17.0%	24.4%
18:0	12.1%	9.7%	9.8%	12.5%	10.0%
20:0	0.3%	0.3%	0.2%	0.1%	0.1%
Total	43%	36%	34%	34%	40%
Monounsaturated					
16:1	9.9%	19.6%	19.4%	13.0%	10.0%
18:1	14.4%	13.5%	14.4%	12.5%	16.0%
20:1	5.3%	2.6%	1.2%	2.0%	4.4%
Total	30%	36%	35%	28%	30%
PUFA (ω6)**					
18:2	2.0%	1.7%	2.6%	3.40%	3.4%
20:2	2.0%	1.1%	1.4%	0.92%	1.9%
20:4	4.9%	7.6%	6.5%	8.5%	8.2%
PUFA (ω3)***					
18:3	2.4%	2.8%	4.0%	3.0%	1.1%
18:4	0.5%	0.3%	0.2%	0.2%	0.5%
20:5	9.7%	8.5%	9.6%	11.8%	7.1%
22:5	1.6%	2.9%	1.5%	3.8%	7.1%
22:6	4.5%	3.4%	5.4%	7.0%	4.7%
Total PUFA	27%	28%	31%	38%	30%

Data are means and expressed as % of total fatty acids.

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

Table 4.14 Concentrations of neutral lipid classes (mg lipid per g tissue dry weight) in muscle of Metapenaeus ensis during ovarian maturation.

	Ovarian maturation stage				
	I	II	III	IV	V
Monoglycerides	0.34±0.07	0.55±0.11	0.27±0.05	0.35±0.08	0.29±0.05
Diglycerides	0.16±0.01	0.12±0.04	0.14±0.02	0.14±0.03	0.15±0.01
Triglycerides	18±6	33±10	47±16	42±3	35±4
Cholesterol	27±13	22±9	27±4	28±9	24±4
Free fatty acids	17±7	20±8	16±8	19±5	42±3
Total neutral lipids	63±6	75±6	90±8	88±7	82±9

Values are means ± SE of tripilcate determinations.

The concentration of total neutral lipids is the sum of the concentrations of neutral lipid classes.

* Values are significantly different (1-way ANOVA; $P < 0.05$).

Table 4.15 Concentrations of polar lipid classes (mg lipid per g tissue dry weight) in muscle of Metapenaeus ensis during ovarian maturation.

	Ovarian maturation stage				
	I	II	III	IV	V
Sphingomyelin	2.6±0.8	2.5±1.4	2.1±0.4	3.3±0.7	2.0±0.2
Phosphatidylcholine	8.6±2.8	10±0.5	10±0.8	8.3±1.2	9.8±0.7
Phosphatidylserine	3.1±0.7	3.8±1.8	3.8±0.9	4.1±1.1	3.3±0.5
Phosphatidylethanolamine	6.6±2.5	5.3±1.4	4.8±1.3	6.5±1.5	6.3±1.4
Total polar lipids	19±3	25±8	22±2	23±10	26±2

Values are means ± SE of triplicate determinations.

The concentration of total polar lipids are determined together with neutral lipids listed in Table 3.4 after separation of lipid classes.

* Values are significantly different (1-way ANOVA; $P < 0.05$).

Table 4.16a Concentrations of muscular fatty acid content during ovarian maturation of Metapenaeus ensis..

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0	1.17±0.02	0.84±0.13	0.87±0.07	0.73±0.07	0.83±0.05
16:0	6.80±0.10	6.50±0.06	7.20±0.07	6.75±0.11	6.50±0.11
18:0*	6.65±0.14	6.95±0.12	6.80±0.03	6.65±0.15	6.65±0.13
20:0	2.64±0.09	2.60±0.07	2.69±0.15	2.68±0.06	2.67±0.10
Total	17.3±0.4	16.9±0.3	17.6±0.3	16.8±0.4	16.6±0.3
Monounsaturated					
16:1	3.11±0.12	3.21±0.08	3.22±0.05	3.36±0.12	3.06±0.27
18:1*	7.55±0.02	7.55±0.05	8.65±0.10	8.20±0.10	14.8±0.2
20:1*	0.47±0.02	0.24±0.01	0.19±0.03	0.19±0.02	0.57±0.02
Total*	11.2±0.2	11.0±0.2	12.1±0.2	11.8±0.3	10.8±0.5
PUFA (ω6)**					
18:2	0.36±0.06	0.33±0.03	0.33±0.01	0.32±0.02	0.35±0.03
20:2*	0.14±0.02	0.13±0.02	0.23±0.04	0.25±0.01	0.20±0.01
20:4*	2.09±0.04	2.56±0.04	2.34±0.10	2.39±0.02	2.46±0.05
PUFA (ω3)***					
18:3	0.25±0.01	0.28±0.02	0.34±0.06	0.26±0.02	0.35±0.03
18:4*	0.48±0.02	0.46±0.02	0.48±0.03	0.34±0.01	0.47±0.03
20:5*	4.72±0.07	4.86±0.15	4.72±0.10	4.81±0.04	4.13±0.05
22:5	0.39±0.04	0.42±0.02	0.43±0.02	0.42±0.03	0.40±0.03
22:6*	4.34±0.11	4.68±0.09	4.56±0.09	5.15±0.02	4.88±0.05
Total PUFA	12.8±0.4	13.7±0.4	13.4±0.4	13.9±0.2	13.2±0.3
Total	41.2±0.9	41.6±0.8	43.1±0.8	42.8±0.6	40.6±1.0

Data are means ± SE of triplicate determinations, and expressed as mg fatty acid/g tissue dry weight.

* Values are significantly different (1-way ANOVA; $P < 0.05$).

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

Table 4.16b Relative amounts of fatty acid content in total lipids of muscle during ovarian maturation of Metapenaeus ensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0	2.8%	2.0%	2.0%	1.7%	2.0%
16:0	16.6%	15.6%	16.7%	15.9%	16.0%
18:0	16.1%	16.7%	15.8%	15.6%	16.4%
20:0	6.4%	6.3%	6.2%	6.3%	6.6%
Total	42%	41%	41%	39%	41%
Monounsaturated					
16:1	7.6%	7.7%	7.5%	7.9%	7.5%
18:1	18.4%	18.1%	20.1%	19.3%	18.2%
20:1	1.1%	0.6%	0.4%	0.4%	0.7%
Total	27%	26%	28%	28%	27%
PUFA (ω6)**					
18:2	0.9%	0.8%	0.8%	0.8%	0.9%
20:2	0.3%	0.3%	0.5%	0.6%	1.1%
20:4	5.1%	0.6%	0.4%	5.6%	6.1%
PUFA (ω3)***					
18:3	0.6%	0.7%	0.8%	0.6%	0.9%
18:4	1.2%	1.1%	1.1%	0.8%	1.1%
20:5	11.5%	11.7%	11.0%	11.3%	10.2%
22:5	0.9%	1.0%	1.0%	1.0%	1.0%
22:6	10.6%	11.3%	10.6%	12.1%	12.0%
Total PUFA	31%	33%	31%	33%	33%

Data are means and expressed as % of total fatty acids.

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

Table 4.17a Concentrations of fatty acid content in muscular neutral lipids during ovarian maturation of Metapenaeus ensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0	1.38±0.08	1.17±0.08	1.24±0.05	1.09±0.06	1.19±0.01
16:0*	5.25±0.40	5.20±0.25	6.15±0.40	5.40±0.05	5.41±0.15
18:0	5.10±0.30	5.61±0.07	5.75±0.20	5.32±0.12	5.53±0.15
20:0	2.62±0.21	2.16±0.08	2.29±0.14	2.14±0.18	2.72±0.07
Total	14.4±0.8	14.0±0.4	15.5±0.7	14.0±0.4	14.8±0.4
Monounsaturated					
16:1	2.89±0.07	3.07±0.04	2.74±0.18	2.69±0.16	3.04±0.09
18:1*	5.80±0.20	6.00±0.25	7.35±0.15	6.55±0.15	6.15±0.15
20:1	0.14±0.03	0.14±0.02	0.16±0.04	0.15±0.01	0.14±0.02
Total	8.9±0.3	9.3±0.4	10.3±0.4	9.4±0.3	9.3±0.3
PUFA (ω6)**					
18:2	0.33±0.07	0.31±0.04	0.33±0.02	0.31±0.01	0.34±0.02
20:2*	0.11±0.01	0.11±0.01	0.19±0.01	0.20±0.02	0.16±0.04
20:4	1.61±0.08	2.05±0.16	1.99±0.08	1.91±0.05	2.04±0.15
PUFA (ω3)***					
18:3	0.19±0.02	0.23±0.02	0.29±0.03	0.21±0.02	0.29±0.03
18:4*	0.37±0.04	0.37±0.02	0.41±0.03	0.27±0.02	0.39±0.02
20:5*	3.63±0.11	3.89±0.12	4.01±0.20	3.85±0.11	3.43±0.08
22:5	0.30±0.02	0.33±0.04	0.37±0.02	0.33±0.03	0.33±0.02
22:6	2.84±0.10	3.24±0.10	3.38±0.17	3.10±0.22	3.09±0.06
Total PUFA	9.4±0.3	10.5±0.3	11.0±0.4	10.2±0.3	10.1±0.3
Total*	32.6±1.6	33.8±1.3	36.6±1.6	33.5±1.3	34.2±1.0

Data are means ± SE of triplicate determinations, and expressed as mg fatty acid/g tissue dry weight.

* Values are significantly different (1-way ANOVA; $P < 0.05$).

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

Table 4.17b Relative amounts of fatty acid content in muscular neutral lipids during ovarian maturation of Metapenaeus ensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0	4.3%	3.5%	3.4%	3.2%	3.5%
16:0	16.1%	15.4%	16.7%	16.1%	15.7%
18:0	15.7%	16.5%	15.8%	15.9%	16.1%
20:0	8.0%	6.2%	6.2%	6.4%	8.0%
Total	44%	42%	42%	42%	43%
Monounsaturated					
16:1	8.9%	9.1%	7.5%	8.0%	8.9%
18:1	17.8%	17.8%	20.1%	19.6%	18.0%
20:1	0.4%	0.4%	0.4%	0.4%	0.4%
Total	27%	27%	28%	28%	27%
PUFA (ω6)**					
18:2	1.0%	0.9%	0.9%	0.90%	1.0%
20:2	0.3%	0.3%	0.5%	0.6%	0.5%
20:4	4.9%	6.1%	5.4%	5.7%	6.0%
PUFA (ω3)***					
18:3	0.6%	0.7%	0.8%	0.6%	1.0%
18:4	1.1%	1.1%	1.1%	0.8%	1.1%
20:5	11.1%	11.5%	10.9%	11.5%	10.0%
22:5	0.9%	1.0%	1.0%	1.0%	1.0%
22:6	8.7%	9.6%	9.2%	9.3%	9.0%
Total PUFA	29%	31%	30%	30%	30%

Data are means and expressed as % of total fatty acids.

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

Table 4.18a Concentrations of fatty acid content in muscular polar lipids during ovarian maturation of Metapenaeus ensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0	0.29±0.01	0.15±0.02	0.09±0.02	0.15±0.02	0.13±0.02
16:0	1.71±0.10	1.11±0.05	0.72±0.09	1.35±0.07	0.97±0.07
18:0*	1.66±0.05	1.18±0.11	0.68±0.10	1.33±0.08	1.00±0.08
20:0	0.40±0.05	0.42±0.04	0.33±0.03	0.37±0.03	0.41±0.03
Total*	4.1±0.2	2.9±0.3	1.8±0.2	3.2±0.2	2.5±0.3
Monounsaturated					
16:1*	0.78±0.05	0.55±0.01	0.32±0.02	0.67±0.04	0.46±0.02
18:1	1.89±0.08	1.28±0.05	1.37±0.09	1.64±0.06	1.11±0.05
20:1	0.02±0.01	0.04±0.01	0.02±0.01	0.04±0.02	0.05±0.01
Total	2.7±0.2	1.9±0.1	1.7±0.1	2.4±0.1	1.6±0.1
PUFA (ω6)**					
18:2	0.09±0.01	0.08±0.01	0.09±0.01	0.06±0.01	0.07±0.01
20:2	0.09±0.01	0.07±0.02	0.08±0.02	0.11±0.02	0.08±0.01
20:4*	0.62±0.04	0.64±0.06	0.74±0.06	0.48±0.03	0.52±0.05
PUFA (ω3)***					
18:3	0.06±0.01	0.05±0.01	0.07±0.02	0.05±0.02	0.05±0.01
18:4	0.12±0.01	0.08±0.01	0.09±0.02	0.06±0.02	0.07±0.01
20:5*	2.18±0.07	1.83±0.08	1.97±0.06	1.96±0.03	1.62±0.06
22:5*	0.10±0.02	0.07±0.01	0.10±0.01	0.09±0.01	0.06±0.01
22:6	1.09±0.06	0.80±0.02	0.96±0.06	1.03±0.09	0.73±0.03
Total PUFA	4.4±0.2	3.6±0.2	4.1±0.2	3.9±0.2	3.2±0.2
Total	11.1±0.5	8.3±0.6	7.6±0.6	9.4±0.5	7.3±0.5

Data are means ± SE of triplicate determinations, and expressed as mg fatty acid/g tissue dry weight.

* Values are significantly different (1-way ANOVA; $P < 0.05$).

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

Table 4.18b Relative amounts of fatty acid content in muscular polar lipids during ovarian maturation of Metapenaeus ensis.

	Ovarian maturation stage				
	I	II	III	IV	V
Saturated					
14:0	2.6%	1.6%	1.1%	1.6%	1.7%
16:0	15.4%	13.3%	9.5%	14.4%	13.3%
18:0	15.0%	14.2%	8.9%	14.2%	13.6%
20:0	3.6%	5.1%	4.3%	3.9%	5.5%
Total	37%	34%	24%	34%	34%
Monounsaturated					
16:1	7.0%	6.6%	4.2%	7.2%	6.3%
18:1	17.1%	15.4%	18.0%	17.5%	15.2%
20:1	0.2%	0.5%	0.2%	0.4%	0.6%
Total	24%	22%	23%	25%	22%
PUFA (ω6)**					
18:2	0.8%	0.9%	1.1%	0.7%	0.9%
20:2	0.8%	0.8%	1.0%	1.1%	1.1%
20:4	5.6%	7.6%	9.7%	5.1%	7.1%
PUFA (ω3)***					
18:3	0.6%	0.6%	0.9%	0.5%	0.7%
18:4	1.1%	0.9%	1.2%	0.7%	1.0%
20:5	19.7%	26.0%	26.0%	20.9%	22.8%
22:5	0.9%	0.8%	1.3%	0.9%	0.8%
22:6	9.8%	9.6%	12.6%	10.9%	10.0%
Total PUFA	39%	44%	53%	41%	44%

Data are means and expressed as % of total fatty acids.

** PUFA (ω6): ω6-type polyunsaturated fatty acids

*** PUFA (ω3): ω3-type polyunsaturated fatty acids

4.4 Discussion

4.4.1 Variation of lipids in ovary during ovarian maturation

As the ovary matured, the gonadosomatic index increased from stage I to IV, and then decreased at stage V. Similar results have been reported in other penaeids, such as P. japonicus (Teshima & Kanazawa, 1983), P. kerathurus (Mourente & Rodriguez, 1991) and Pleoticus muelleri (Jeckel et al., 1989). The maximum body weight is found at stage IV at which the gonadosomatic index (GSI) was the highest. This correlation suggests the increase in dry weight at stage IV may be due to an increase in ovary weight. This phenomenon has also been noted in P. japonicus (Teshima & Kanazawa, 1983) and P. kerathurus (Mourente and Rodriguez, 1991).

Expressed in the same unit as in this study, the concentrations of ovarian lipids in P. monodon and P. kerathurus increase from 6% and 12% of tissue dry weight at stage I to 16% and 25% at stage IV, respectively. The concentration of ovarian lipids in Metapenaeus ensis ranged from 22% to 43% of tissue dry weight. Thus, the concentration of ovarian lipids in M. ensis is about 40% higher than Penaeus monodon (Millamena & Pascual, 1990), P. kerathurus (Mourente & Rodriguez, 1991) and P. chinensis (see Chapter 3). The tremendous increase in ovarian lipids during ovarian maturation suggests the significance of

lipids in this process. Similar results have been documented in other shrimps, such as Penaeus duorarum (Gehring, 1974), P. japonicus (Teshima & Kanazawa, 1983), P. aztecus, P. setiferus (Castille & Lawrence, 1989), P. monodon (Millamena & Pascual, 1990), P. kerathurus (Mourente & Rodriguez, 1991), and Pleoticus muelleri (Jeckel et al., 1989). The ovarian lipid concentration in these shrimps increases approximately two folds during ovarian maturation, and drop markedly after spawning.

Similar to the ovarian lipids, both neutral and polar lipids in the ovary increased during ovarian maturation of M. ensis (Tables 4.4 & 4.5). This result is consistent with other studies in ovarian lipids in shrimps, such as P. duorarum (Gehring, 1974), P. japonicus (Teshima & Kanazawa, 1973, P. indicus (Galois, 1984), P. kerathurus (Mourente & Rodriguez, 1991), and Pleoticus muelleri (Jeckel et al., 1989). The concentration of neutral lipids in M. ensis is higher than that of polar lipids. The ratio of neutral lipid to polar lipid (NL/PL) varied between 1.3 to 2.0. This result agrees well with the results in P. japonicus (Teshima & Kanazawa, 1983) and the polar shrimp, Chorismus antarcticus (Clarke, 1977). The NL/PL ratio of P. japonicus ranges from 1 to 1.6 and that of Chorismus antarcticus is 1.1. However, our results are in contrast to those reported in P. duorarum (Gehring, 1974), P. indicus (Galois, 1984), P. kerathurus (Mourente & Rodriguez, 1991) and Pleoticus muelleri (Jeckel et al.,

1989). The NL/PL ratios are reported to be 0.21 to 0.34 in P. duorarum, to be 0.25 to 0.50 in P. indicus, to be 0.44 to 0.97 in P. kerathurus, and to be 0.3 to 0.5 in Pleoticus muelleri.

Among neutral lipid classes in the ovary of M. ensis, triglycerides, cholesterol and free fatty acids, which comprised more than 90% of neutral lipids, were dominant. There were marked increases in triglycerides and cholesterol during ovarian maturation. Triglycerides accumulated is believed to be used for energy reserves in eggs for early larval development of shrimp larvae (Fraser, 1989). The concentration of triglycerides in mature ovary is found to be about 20% lower in M. ensis than in Penaeus chinensis (see Chapter 3). This difference may be attributed to the different spawning seasons of these two shrimps. In Hong Kong waters, M. ensis spawns in summer while Penaeus chinensis spawns in winter. Therefore, the eggs of P. chinensis may have to contain more triglycerides as energy reserves for a longer period of pre-feeding larval development.

The concentration of cholesterol in the mature ovary of M. ensis is about 30% higher than that reported in P. kerathurus (Mourente & Rodriguez, 1991) and P. chinensis (See Chapter 3). Higher concentration of ovarian cholesterol in M. ensis may reflect a greater demand of cholesterol in ovarian development in M. ensis than in these two penaeids.

In mature ovary, the concentrations of free fatty acids in M. ensis are 60% lower than in P. chinensis. Since free fatty acids are metabolic products, our data suggest that less free fatty acids are released from acylglycerides and phospholipids in M. ensis for energy production or biosynthesis.

Unlike in P. chinensis where phosphatidylcholine alone is responsible for the changes in ovarian polar lipids, both phosphatidylcholine and phosphatidylethanolamine, the dominant polar lipids in M. ensis, are responsible for the changes of the ovarian polar lipids. Similar results were reported in P. duorarum (Gehring, 1974), P. japonicus (Teshima et al., 1988) and P. kerathurus (Mourente and Rodriguez, 1991). As a whole, cholesterol, triglycerides and polar lipids are responsible for the lipid accumulation in the ovary of M. ensis.

The ovarian lipids of M. ensis contain high levels of palmitic (16:0), palmitoleic (16:1), oleic (18:1) and eicosapentaenoic (20:5ω3) acids. The increases of these acids during ovarian maturation indicates that these acid are required in oocyte production. Guary et al. (1974) have demonstrated the presence of high levels of saturated and monounsaturated fatty acids in the ovary of P. japonicus and postulated the necessity of these acids for building up triglycerides as energy reserves for embryonic and early larval development.

It has been assumed that polyunsaturated fatty acids are involved to some extent in the reproductive process of penaeids (Brown et al., 1979; Lawrence et al., 1980). A large reduction in polyunsaturated fatty acids of the ovary from stage IV to V suggests the spawned eggs contain a large amount of polyunsaturated fatty acids (Table 4.6a). Clarke (1977) has noted that the concentration of polyunsaturated fatty acids is high in eggs of polar shrimp, Chorismus antarcticus, comprising 45% of total fatty acids in egg polar lipids and 47% of total fatty acids in egg triglycerides. In the eggs of the crab, Pachygrapsus marmoratus, polyunsaturated fatty acids also constitute about 30% of total fatty acids (Lautier & Lagarrigue, 1988). These acids would constitute important components of the cell membrane of the eggs.

4.4.2 Variation of lipids in hepatopancreas during ovarian maturation

When expressed as % tissue dry weight, the lipid concentration in the hepatopancreas M. ensis is lower than those in P. kerathurus (Mourente & Rodriguez, 1991) and P. chinensis (See Chapter 3), but higher than that in P. monodon (Millamena & Pascual, 1990). The difference may be related to species differences, for example, in the diets. The concentration of hepatopancreatic lipids in M. ensis decreases from 40 mg/g of tissue dry weight at stage I to

19 mg/g at stage V (Table 4.2). This pattern agrees with the changes of hepatopancreatic lipids in the shrimp, Penaeus setiferus (Castille & Lawrence, 1989) and the crab, Pachygrapsus marmoratus (Lautier & Lagarrigue, 1988), but differs from the patterns in the shrimps, such as Penaeus japonicus (Teshima & Kanazawa, 1983), P. monodon (Millamena & Pascual, 1990), P. kerathurus (Mourente & Rodriguez, 1991) and P. chinensis. In these shrimps, the lipids are accumulated in hepatopancreas during the immature and developing stages and become depleted in late mature stages. The increase in hepatopancreatic index of M. ensis during ovarian maturation despite a decrease in lipid concentration may be due to an increase in the concentration of biochemical components other than lipids, such as carbohydrate and protein, in the hepatopancreas. An accumulation of carbohydrates or proteins in the hepatopancreas during ovarian maturation has been reported in P. setiferus (Castille & Lawrence, 1989).

Similar to the changes of total hepatopancreatic lipids, both neutral and polar lipid concentrations decreased from stage I to IV (Tables 4.9 & 4.10). This pattern of decrease agrees with the result in P. indicus (Galois, 1984), but differs from other penaeids, such as P. japonicus (Teshima & Kanazawa, 1983), P. monodon (Millamena & Pascual, 1990), P. kerathurus (Mourente & Rodriguez, 1991) and P. chinensis. In these shrimps, the concentrations of neutral polar and neutral lipids in M.

ensis increased from immature stage to a maximum at developing stage, then decreased to low level at mature stage.

Triglycerides, cholesterol and free fatty acids, which comprised more then 90% of neutral lipids in the hepatopancreas of M. ensis, are the major components of neutral lipids. Among them, triglycerides are mostly responsible for the changes in neutral lipids. Lower concentration of triglycerides is found in M. ensis than in P. chinensis. This difference may also be attributed to differences in food sources of the two species. Unlike in insects, triglycerides in decapod crustaceans are transported in the form of polar lipids, rather than diglycerides (Gilbert & O'Connor, 1970). Thus, the concentration of diglycerides in decapods would be much lower than that of triglycerides.

The concentration of free fatty acids in the hepatopancreas of M. ensis is not as high as in P. chinensis. The reason of this difference is unknown. The concentrations of triglycerides and free fatty acids increased after spawning, probably as a result of the reabsorption of the nutrients from the residual eggs of incomplete spawning.

All polar lipid classes in the hepatopancreas exhibited a decrease in concentration along ovarian maturation. This decrease may be related to the transport of lipids from the hepatopancreas to ovary (see section

4.3.4). The concentration of polar lipids in M. ensis was about 30% lower than that in P. chinensis. Phosphatidylcholine and phosphatidylethanolamine are responsible for changes in polar lipids.

Since shrimps, like other decapod crustaceans, are incapable of de novo synthesis of polyunsaturated fatty acids and are exclusively dependent upon exogenous sources for these acids (D'Abramo & Lovell, 1991), the increase of polyunsaturated fatty acids in the hepatopancreas may result from ingesting food which contains high levels of these acids. Accumulation of the polyunsaturated fatty acids in total lipids, neutral and polar lipids in the hepatopancreas during ovarian maturation appeared to strengthen the theory that polyunsaturated fatty acids are necessary for vitellogenesis of penaeids (Middleditch et al., 1980).

4.4.3 Variation of lipids in muscle during ovarian maturation

The lipids in abdominal muscle, which constituted less than 5% of tissue dry weight, was much lower in concentration than the lipids in the ovary and hepatopancreas. The concentrations of lipids in muscle remained roughly constant during ovarian maturation. Similarly, the concentrations of both neutral and polar lipids in muscle were much lower than those in the ovary

and hepatopancreas (Tables 4.13a & 4.15a). The changes of concentrations of both neutral and polar lipids were limited when compared to those in the ovary and hepatopancreas. The concentrations of neutral lipids in muscle were similar in both M. ensis and P. chinensis, but M. ensis exhibits a lower concentration of polar lipids than P. chinensis (See Chapter 3).

The concentrations of fatty acids in total lipids, neutral and polar lipids of muscle were much lower than those in the ovary and hepatopancreas and remained roughly constant during ovarian maturation. There was no decrease of fatty acids in polar lipids during ovarian maturation as observed in Penaeus chinensis. This difference may be attributed to a lower demand of polar lipids in M. ensis or an adequate supply of polar lipids from the diets during ovarian maturation.

4.4.4 Mobilization of lipids during ovarian maturation

During ovarian maturation, the concentration of total lipids in ovary of Metapenaeus ensis increased two folds while the concentration of lipids in hepatopancreas decreased about 50%. These results indicated that there was a mobilization of lipids from the hepatopancreas to ovary during ovarian maturation. Mobilization of the lipids during ovarian maturation has been documented in other decapod crustaceans, such as the shrimps,

Parapenaeopsis hardwickii (Kulkarni & Nagabhushanam, 1979), Penaeus indicus (Galois, 1984), P. japonicus (Teshima & Kanazawa, 1983), P. monodon (Millamena & Pascual, 1990), and P. kerathurus (Mourente & Rodriguez, 1991), and the crabs, Paratelphusa hydrodromous (Adiyodi & Adiyodi, 1971), Carcinus maenas (Paulus & Laufer, 1982), and Pachygrapsus marmoratus (Lautier & Lagarrigue, 1988). Lipids stored in the hepatopancreas are transported to the ovary in order to fulfil the great energy demand of the ovary for vitellogenesis.

The absolute amount of ovarian lipids increased about 278 mg per shrimp from stage I to stage IV (Table 4.3). During the same period, the absolute amount of hepatopancreatic lipids decreased by about 42 mg per shrimp (Table 4.3). The amount of decrease in hepatopancreatic lipids was only 17% of the increment of ovarian lipids in M. ensis. As shown in Penaeus stylirostris (Bray et al., 1990), the lipids in the ovary increase tremendously during ovarian maturation, and the hepatopancreas has a limited storage capacity. The lipid mobilized from the hepatopancreas to the ovary may depend on immediate ingestion of dietary lipids, rather than on stored lipids in the hepatopancreas alone (Harrison, 1991).

Concentrations of different lipid classes in ovary and hepatopancreas changed similarly as the trend of total lipid concentration during ovarian maturation of M. ensis. From the immature stage to mature stage, the concentration

of neutral lipids increased three folds in ovary while the neutral lipids decreased about 50% in hepatopancreas. Triglycerides, cholesterol and free fatty acids were responsible for the changes of neutral lipids in these two organs during maturation. Similar results have also reported in other penaeids (Teshima & Kanazawa, 1983; Galois, 1984; Mourente & Rodriguez, 1991). Mobilization of triglycerides and cholesterol may indicate the importance of these substances during ovarian maturation. Triglycerides are necessary to be stored in eggs for energy reserves during embryonic and pre-feeding larval development while cholesterol is one of the essential components of cell membrane.

During ovarian maturation, the concentration of polar lipids increased four folds in the ovary of M. ensis where the concentration of polar lipids decreased about 40% in hepatopancreas. Similarly, the concentration of phosphatidylcholine and phosphatidylethanolamine, the dominant components of polar lipids, also exhibited an increase in ovary and a decrease in hepatopancreas during maturation. This phenomenon agrees with the results in P. japonicus (Teshima & Kanazawa, 1983) and P. kerathurus (Mourente & Rodriguez, 1991). Polar lipids are essential in the eggs because they are the components of cell membrane and vitellin, the major constituent of yolk.

The increase in concentrations of saturated and monounsaturated fatty acids in ovary with a concurrent

decrease of these acids in hepatopancreas was also found during ovarian maturation in M. ensis. These results suggested the possible movement of fatty acids from hepatopancreas to ovary during maturation. The mobilization of fatty acids has been reported in the shrimps, such as P. japonicus (Teshima & Kanazawa, 1983) and P. kerathurus (Mourente & Rodriguez, 1991), and the crabs, such as Paratelphusa hydrodromous (Adiyodi & Adiyodi, 1971) and Pachygrapsus marmoratus (Lautier & Lagarrigue, 1988). The variation of these acids may be related to changes of triglycerides and polar lipids during ovarian maturation because these acids are always incorporated into triglycerides and polar lipids.

In this study, the changes of lipid concentrations in ovary and hepatopancreas during maturation suggest the occurrence of mobilization of lipids from hepatopancreas to ovary. This mobilization during ovarian maturation was confirmed by a large increase of total lipids in haemolymph in the shrimp Penaeus japonicus (Teshima & Kanazawa, 1983) and the crab Pachygrapsus marmoratus (Lautier & Lagarrigue, 1988). Lipids in crustaceans are transported by lipoproteins, which are rich in polar lipids. Cholesterol in hepatopancreas is associated and then transported by lipoprotein to ovary (Teshima & Kanazawa, 1980; Chang & O'Connor, 1983). Triglycerides are converted into polar lipids in the hepatopancreas for export into the haemolymph

as components of high density lipoproteins (HDL) (Harrison, 1990). Afterwards, the lipoproteins are rapidly transported from the haemolymph to the ovary and the components of lipoproteins are released inside the ovary. Part of polar lipids is reconverted to triglycerides. These triglycerides are either be used for energy production during the manufacture of oocytes, or be stored as fat droplets in oocytes. The appearance of lipid globules has been reported in the ovary of shrimps, such as Crangon crangon, several species of Palaemonetes (Beam & Kessel, 1963), Penaeus japonicus (Yano, 1988), P. monodon (Tan-Fermin & Pudadera, 1989), and the crayfish Orconestes species (Beam & Kessel, 1963). Part of polar lipids, as well as cholesterol is used for membrane construction as size and number of the oocytes increase during ovarian maturation. Certain amount of polar lipids is also present in the yolk.

Chapter 5 General conclusions

Major results on the variation of lipid composition in Penaeus chinensis and Metapenaeus ensis during ovarian maturation are summarized as follows:

a. As the gonadosomatic index increases during ovarian maturation, lipids are accumulated in the ovaries.

b. Polar lipids, cholesterol and triglycerides are the major components of ovarian lipid, comprising about 70% of total lipids, and are responsible for the increase in ovarian lipid.

c. Phosphatidylcholine and phosphatidylethanolamine are the dominant ovarian polar lipids, constituting about 50% of polar lipids. They are the major components which are responsible for changes in ovarian polar lipids.

d. The ovarian lipids contain high levels of palmitic (16:0), palmitoleic (16:1), oleic (18:1) and eicosapentaenoic (20:5ω3) acids, comprising 70% of total fatty acids. The large increases in these acids during ovarian maturation indicate that these acids are required for oocyte production.

e. Hepatopancreatic lipids account for 20 to 40% of tissue dry weight and decrease during ovarian maturation.

f. The major components of hepatopancreatic lipids are triglycerides and polar lipids, comprising about 60% of total lipids. They exhibit a decrease during ovarian maturation.

g. Phosphatidylcholine and phosphatidylethanolamine are the dominant polar lipids in hepatopancreas, accounting for about 30% of polar lipids, and their concentrations decrease during ovarian maturation.

h. The hepatopancreatic lipids contain high levels of palmitic (16:0), palmitoleic (16:1), oleic (18:1) and eicosapentaenoic (20:5ω3) acids, comprising about 60% of total fatty acids. The concentrations of saturated and monounsaturated fatty acids decrease during ovarian maturation while that of polyunsaturated fatty acids increases.

i. Accumulation of total lipids, lipid classes and fatty acids in the ovary during ovarian maturation are concurrent with the decrease in hepatopancreatic lipids in P. chinensis and M. ensis. This result indicates that the lipids are mobilized from the hepatopancreas to the ovary for oocyte maturation.

j. The decrease in the absolute amount of hepatopancreatic lipids only accounts for about 20% of the increment in ovarian lipids. Thus, lipid requirement of the ovary depends not only on the lipid stored in the hepatopancreas, but also immediate ingestion of dietary lipids during ovarian maturation.

k. The concentrations of total lipid, lipid classes and fatty acids in muscle are much lower than those in the ovary and hepatopancreas and remain relatively constant during ovarian maturation, suggesting that the muscle lipids did not play an important role in ovarian maturation.

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